MOLECULAR CHARACTERIZATION OF WETLAND SOIL BACTERIAL COMMUNITY IN CONSTRUCTED MESOCOSMS

THESIS

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Abstract

The use of wetlands to effectively remediate aquifers contaminated with chlorinated solvents is an emerging technique, which shows high promise. In order to better understand this process and test its legitimacy, a treatment wetland was constructed at Wright-Patterson AFB, Dayton, Ohio and, in a joint effort with Wright State University (WSU), has previously shown the effective removal of PCE. The purpose of this research was to characterize the soil bacterial community, pre-PCE injection, among three wetland plant species from the sedge family (Cyperaceae) within constructed wetland mesocosms and to identify any bacterial dominance.

Carex comosa, *Scirpus atrovirens*, and *Eleocharis erythropoda* were planted in multiple columns (mesocosms) filled with inoculated soils; water flow was through a vertical up-flow design representative of a ground water-fed wetland. DNA extractions were made from soil samples taken at each of three depths. 16S rDNA libraries were constructed to characterize the bacterial communities in mesocosms for each plant, to use for comparative analyses of the effects each plant might have on microbial community structure. BLAST and RDP-II's Classifier programs were used to classify the sequences in the libraries. A total of 396 sequences were attained, ultimately resulting in 300 unique accession numbers. Eleven phyla were represented by 177 classifiable clones. A variety of diversity indices were used to show an extremely high species richness, indicating that further sequencing is needed to determine phylotype dominance, if any exists, within the columns. This study is a first step in understanding the role of wetland plant-associated microbial communities in remediation of chlorinated solvents.

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Ethan C. Bishop

To My Family

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MOLECULAR CHARACTERIZATION OF WETLAND SOIL BACTERIAL COMMUNITY IN CONSTRUCTED MESOCOSMS

I. Introduction

The intentional construction of wetlands to remove environmental contaminants is a relatively new technology. The term "constructed wetland" is used to define those wetlands that are built expressly for the purposes of water quality treatment (Kadlec and Knight, 1996). In 1973, the first intentionally engineered, constructed wetland treatment systems in North America were constructed to remove contaminants from stormwater run-off and municipal run-off. Since then, wetlands have also been designed and constructed to treat process waters from industry (Kadlec and Knight, 1996) and are being used more and more as a viable bioremediation technique. However, the relationship between rooted plant species and bacterial communities within these systems has received little attention.

While advances in bioremediation techniques have increased tremendously over the past decade, so too has the development of molecular microbiology. Using nucleic-acid analysis, one can now determine bacterial dominance, diversity, distribution, genetic capabilities, and bacterial phylogeny. This greatly enhances the capability and research tools needed to gain a better understanding into microbial ecology, biogeochemical fate, and treatment of contaminants such as tetrachloroethylene (PCE).

The purpose of this research was to characterize bulk soil bacterial community among three wetland plant species from the sedge family (Cyperaceae) within a constructed reductive dechlorination wetland and to identify any bacterial dominance prior to PCE inoculation. This information will further the understanding of the processes of in situ bioremediation and the use of wetlands as a viable technique for removing halogenated organic contaminants from

subsurface water. It may ultimately serve to identify additional species linked to the dehalogenation process.

Carex comosa, *Scirpus atrovirens*, and *Eleocharis erythropoda* were planted in separate upward flow columns (mesocosms), inoculated with soil from the constructed treatment wetland cells at Wright-Patterson Air Force Base (WPAFB), and continuously fed PCE contaminated water at a concentration representative of the on-site contaminated aquifer. Prior to the addition of PCE into the water flow and following the establishment of each of the plantings, DNA analysis using polymerase chain reaction (PCR), cloning, and sequencing was used to characterize the soil bacterial community among these plantings and to qualify any relations between the various plant species at three depths. Later analysis will be completed to examine rhizoplane and bulk soil bacterial communities, post-PCE inoculation. Separate, yet simultaneous, research will be used to substantiate the findings by examining the concentrations of chlorinated solvents at multiple depths within the mesocosms, as well as comparing DNA analysis taken seasonally from field samples from both contaminated and uncontaminated sites at similar depths. The study represents a joint effort between students and faculty of the Air Force Institute of Technology (AFIT) and Wright State University.

Background

Chlorinated solvents have been shown to be among the most common water table contaminants in the United States. Of the 1,636 sites currently listed under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) National Priority List (NPL), volatile organic compounds (VOCs), in particular, chlorinated solvents, showed the greatest frequency of occurrence (NRC, 1994; U.S. Department of Health and Human Services. ATSDR, 2003) (See Table 1). The EPA (2004) reports that 69 percent of the sites are contaminated with halogenated VOCs. Similarly, halogenated VOCs are by far the most

common contaminant at Resource Conservation and Recovery Act (RCRA) sites, found at 60 percent of the sites (U.S. EPA, 2004).

Table 1. Chlorinated VOCs Frequencies of Occurrence

NPL Ranking	Name	NPL Site Frequency
4	Vinyl chloride (VC)	608
16	Trichloroethylene (TCE)	1021
30	Tetrachloroethylene (PCE)	930
43	Carbon Tetrachloride	422
87	1,2-Dichloroethane	599
148	1,1,2,2-Tetrachloroethane	327
163	1,1,2-Trichloroethane	274
175	1,2-Dichloroethene, Trans-	598
213	1,2-Dichloroethylene	450
277	1,2-Dichloroethene, Cis-	263
282	Dichloroethylene (DCE)	114

Source: (U.S. Department of Health and Human Services. Agency for Toxic Substances and Disease Registry, 2003). Ranking based on combination of toxicity, frequency, and potential for human exposure.

Chlorinated solvents were produced in large quantities and widely used in a multitude of applications and operations including the decaffeination of coffee, pet food production, pharmaceuticals, cosmetics, dry-cleaning fabrics, and metal degreasing operations following WWII; however, their use was highly curtailed in the late 1970's when they became suspected carcinogens (Chapelle, 2001). This study focuses on the chlorinated aliphatic (straight-chained) compound tetrachloroethylene (PCE), also known as perchloroethylene (Figure 1), and the associated bacteria responsible for degradation of PCE to its reduced products of trichloroethylene (TCE), dichloroethylene (DCE), vinyl chloride (VC), and finally ethene.

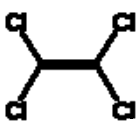


Figure 1. Tetrachloroethylene (PCE).

Prior to the enactment of RCRA in 1980, disposal of chlorinated solvents were unregulated. Ultimately, haphazard disposal resulted in groundwater contamination due to the solvents' physical and chemical properties (U.S. EPA, 2004). Chlorinated solvents have low solubilities and are denser than water, thus fall into the class of contaminants commonly known as dense nonaqueous-phase liquids (DNAPLs). DNAPLs tend to penetrate water-saturated ground, form subsurface pools when they encounter impermeable layers, and subsequently form slow moving plumes within aquifers (Masters, 1997). These migrating plumes can pose serious threats to drinking water supplies where communities rely on groundwater. The listed log K_{ow} and K_{oc} of these compounds predict a moderate hydrophobicity and soil sorption potential; while the Henry's Constants describes the high tendency towards volatilization when exposed to the atmosphere.

Table 2. Physiochemical Properties of Common Chlorinated Solvents

Chlorinated Hydrocarbon	Molecular Weight (g/mol)	Solubility (mg/L)	Vapor pressure (atm)	Henry's Law Constant (atm-m^3/mol)	Soil Sorption Coefficient (log K_{oc})	log K_{ow}	Density (g/ml)
PCE (C_2Cl_4)	165.83	$1.5*10^{2}$ [c]	$2.51*10^{-2}$ [b]	$1.53*10^{-2}$ [b]	2.56[c]	2.88[b]	1.63[a]
TCE (C_2HCl_3)	131.39	$1.1*10^{3}$ [c]	$9.77*10^{-2}$ [b]	$1.07*10^{-2}$ [b]	2.1[c]	2.42[b]	1.46[a]
1,1 DCE ($C_2H_2Cl_2$)	96.94	$4.0*10^{2}$ [c]	$7.86*10^{-1}$ [b]	$3.0*10^{-2}$ [c]	1.81[c]	2.13[b]	1.22[a]
trans-1,2 DCE ($C_2H_2Cl_2$)	96.94	$6.3*10^{3}$ [c]	$4.29*10^{-1}$ [c]	$6.60*10^{-3}$ [c]	1.77[c]	2.06[c]	1.26[a]
cis-1,2 DCE ($C_2H_2Cl_2$)	96.94	$3.5*10^{3}$ [c]	$2.63*10^{-1}$ [c]	$3.37*10^{-3}$ [c]	1.69[c]	1.86[b]	1.28[a]
VC (C_2H_3Cl)	62.5	$1.1*10^{3}$ [c]	$2.57*10^{-1}$ [b]	$5.68*10^{-2}$ [b]	0.9138[c]	0.6 [b]	0.91[a]

Source: a=CRC (2004); b= Schwarzenbach, Gschwend, & Imboden (1993); c= Mackay, Shiu, & Kuo (1993). Note: All properties, except specific gravity, were calculated at 25ºC, 1 atm. Density calculated at 20°C.

The main effects of PCE in humans are neurological, liver, and kidney effects following acute (short-term) and chronic (long-term) inhalation exposure (U.S. Environmental Protection

Agency, 1988). Epidemiological studies of dry-cleaners occupationally exposed to tetrachloroethylene suggest increased risks for several types of cancer. Animal studies have reported an increased incidence of liver cancer in mice, via inhalation and gavage (experimentally placing the chemical in the stomach), and kidney and mononuclear cell leukemia in rats (U.S. EPA, 1988).

The reductive by-products of PCE have been shown to pose risks in the limited studies conducted. TCE is the most common organic water contaminant and is classified as a possible human carcinogen (Hageman, Istok, Field, Buscheck, & Semprini, 2001); DCE is not a listed carcinogen, however, it has been shown to cause decreased red blood cell numbers in animals and affects on the liver and the heart; vinyl chloride is the most toxic of chlorinated solvents and is a known human carcinogen (Masters, 1997). VC is widely distributed contaminant and is a significant intermediate product of reductive dehalogenation of polychlorinated ethenes under anaerobic conditions (Bradley & Chapelle, 1996).

Current EPA guidelines regard PCE contaminant levels above 5.0 ppb as the maximum contaminant level (MCL) for acceptable risk in drinking water. Table 3 lists MCLs for other chlorinated ethenes. The solubilities are several orders of magnitude greater than current drinking water standards, thereby preventing dilution by hydrodynamic dispersion from being a viable mechanism for managing contaminated sites (National Research Council, 1997).

Table 3. Selected Regulatory Limits.
Source: (U.S. Environmental Protection Agency, Revised July 1, 2002)

Compound	MCL (mg/L)
Vinyl chloride (VC)	0.002
cis-dichloroethene	0.07
trans-dichloroethene	0.1
1,1-dichloroethene	0.007
Trichloroethylene (TCE)	0.005
Tetrachloroethylene (PCE)	0.005

Current Treatment Technologies

Over the past few decades the use of PCE in industry has declined more than 80 percent (U.S. EPA, 2004); however cleanup of these solvents remains a significant environmental challenge. The EPA currently estimates the number of contaminated sites requiring clean-up nationwide to be in the range of 235,000 to 355,000 requiring an estimated cost of $170-250 billion (U.S. EPA, 2004). Some clean-up estimates have even ranged as high as $1 trillion (National Research Council (NRC)., 1994).

There is promise in new innovative remediation technologies, but their use is still limited. In 1996 the EPA reported that conventional pump-and-treat systems were employed in 93 percent of all Superfund sites and was combined with *in situ* treatment in only 5 percent of the 603 sites for which clean-up remedies had been selected. At the time, only 9 sites were using *in situ* treatment alone (U.S. EPA, 1996). As of 2004, 851 Superfund sites were being treated; pump-and-treat alone was used in 65 percent and in combination with other treatments at 84 percent of the Superfund sites. *In situ* alone was being used at 31 sites or in combination with other technologies at 135 sites (16 percent). Monitored natural attenuation (MNA) was employed at 201 (24 percent) of the sites (U.S. EPA, 2004). This shows the growing trend towards alternative, more cost effective treatments.

Attaining the most efficient and cost effective treatment technology has been a challenge since CERCLA was initiated; hence many innovative and established forms of remediation technology have been developed to treat this hazard. The term "treatment technology" refers to "any unit operation or series of unit operations that alters the composition of a hazardous substance or pollutant or contaminant through chemical, biological, or physical means so as to reduce toxicity, mobility, or volume of the contaminated materials being treated" (40 CFR. §300

(U.S. Environmental Protection Agency, Revised July 1, 2003)). The following are brief descriptions of varying treatment methods:

Source Control: The use of technologies such as soil vapor extraction, solvent extraction, phytoremediation, chemical treatment, etc. to physically control the introduction of contaminant into the environment.

Pump-and-Treat: Pumping of contaminated water to surface for treatment. Treatment types include air stripping, ion exchange, membrane filtration, or bioremediation techniques.

In Situ Treatment: Treatment within the aquifers themselves by using technologies such as air sparging, phytoremediation (also source control), and permeable reactive barriers. These technologies are designed to separate contaminants from geologic materials in the subsurface, mobilize them into the groundwater or air in soil pores, and extract them from the subsurface.

Containment: Using impermeable subsurface barriers to contain contaminated region. Hydraulic pumping is also used to contain contaminants.

Bioremediation/Natural Attenuation: The reliance on natural attenuation processes to achieve site specific objectives. The goal of bioremediation is to biologically convert a hazardous contaminant such as PCE, TCE, or VC to an innocuous end product. For example, VC can be converted into ethylene, carbon dioxide and water under the proper environmental and biological conditions (Bradley and Chapelle, 1996). Examples of biological reaction technologies include biostabilization, composting, treatment wetlands, and enhanced, in situ bioremediation.

In practice, natural attenuation has several other names, such as intrinsic remediation, intrinsic bioremediation, or passive bioremediation. This natural attenuation can often be the dominant factor in the fate and transport of contaminants such as PCE and TCE. Advantages of natural attenuation include: 1) contaminants are ultimately transformed into relatively innocuous byproducts such as carbon dioxide, ethene, and water, 2) natural attenuation is non-intrusive and

allows for continued use of land and local facilities during remediation, and 3) natural attenuation is less costly than currently available remediation technologies such as pump-and-treat. Disadvantages of natural attenuation include: 1) natural attenuation is subject to natural and manmade changes in local hydrogeologic conditions that may affect contaminant removal, 2) time frames for complete remediation may be relatively long, and 3) intermediate products of bioremediation (e.g. vinyl chloride) may be more toxic than the original contaminant (Wiedemeier, Swanson, Moutoux, Wilson, Kampbell, Hanson, & Haas, 1997 from (Opperman, 2002)).

Pump-and-treat technology has been the method of choice for treatment of the majority of sites at an average cost per volume of treated water of $312 per 1,000 gallons per year (U.S. EPA, 2001). The associated high cost of pump-and-treat systems has led to increased research and development in the field of natural attenuation methods.

Research Objectives

The primary objectives of this research were to:

1. Characterize the pre-PCE injection species diversity of bacteria in mesocosms designed to model constructed, dechlorinating, treatment wetlands and dominant microbial species, if any;
2. Determine correlation, if any, between bulk soils of three wetland plant species and microbial dominance;
3. Determine the effects of soil depth with regards to microbial dominance.

The results may reveal useful symbiotic relationships between wetland soils dominated by a particular species of plants and anaerobic, dehalorespiring bacteria within the soil itself. This information will be very useful when designing and constructing efficient treatment wetlands.

Research Focus and Limitations

This research focuses on the accurate characterization of soil bacterial communities through DNA analysis. It is limited to the identification of microorganism already present in the inoculum taken from treatment wetland constructed on WPAFB and soil used in mesocosm construction taken from Beavercreek Wetlands, Fairborn, Ohio. PCE contamination will be simulated through the continuous injection of PCE into water flow, and its effects evaluated by separate research. Water temperature may not be representative of field conditions.

The chosen methodology involving DNA extraction and polymerase chain reaction (PCR) amplification also introduce unique biases. Efficient DNA extraction is dependent on the methods chosen for cell lysis (i.e. mechanical, sonic, or chemical), DNA sorption to soil particles, and coextraction of humic acids. PCR amplification is dependent on primer choice/design. Some other limitations of using PCR for microbial identification include a difficulty in phylogenetic placement of sequences due to the use of primers generating too short a fragment of a gene, leading to inaccurate or low confidence phylogenies (Rochelle, 2001). PCR can also result in amplified "artifacts" that do not reflect the original template due to point mutations or the formation of chimeric sequences, which are recombinant DNA sequences of two or more different parent sequences in the sample (Hugenholtz & Goebel, 2001). Factors which are thought to result in this phenomenon include using degraded template DNA and excessive cycling (Hugenholtz & Goebel, 2001).

Additionally, this study is limited to one complete assay of DNA present following the establishment of the rooted vegetation due to available time and prohibitive costs associated with PCR, cloning, and sequencing. Other limitations include the choice of PCR primers, which may or may not allow for a complete representation of all microbes present in assays.

II. Literature Review

Overview

The goal of this continuing bioremediation study is to completely characterize the biogeochemical processes involved in the conversion of the hazardous contaminant PCE to its innocuous end products within a constructed dehalogenating wetland. Many in-situ bioremediation remedies already rely on the mechanisms of biodegradation to treat chlorinated solvents. Both aerobic and anaerobic microorganisms are capable of using contaminants as sources of carbon and energy for growth, or as cometabolic substrates that do not contribute to growth, and thus are the driving force behind the remediation of chlorinated solvents.

Remediation with Treatment Wetlands

Wetlands are characterized by the presence of water, continuously or seasonally, either at the surface or within the root zone of the wetland plants. This condition, in turn, results in an environment where plant species dependent on aerobic soils, are absent due to the saturated soils. Wetlands' lower dissolved oxygen levels result in the accumulation of organic matter in wetland soils because of a reduced level of microbial activity and organic decomposition which requires oxygen (Kadlec & Knight, 1996). Their upslope limits are distinguished by a period of saturation which is typically less than 7 to 30 days. Their downslope limits are distinguished by standing water to a depth or duration where, emergent, rooted plants will not survive (typically a depth of 1-2 meters); (Kadlec & Knight, 1996).

Microbial biodegradation and phytoremediation are two major mechanisms by which wetlands contribute to the elimination/transformation of groundwater contaminants. Biodegradation, as previously mentioned, relies on microorganisms to reduce, remove, or stabilize harmful contaminants to their more innocuous forms. Phytoremediation uses plants to remove, transfer, stabilize or destroy contaminants in soil, sediment, and groundwater through

rhizosphere biodegradation, phytoextraction, phytodegradation, and phytostabilization (Clemmer, 2003).

Two treatment wetland cells were, previously, constructed at WPAFB for the purpose of studying the removal of chlorinated solvent contamination from groundwater via biogeochemical processes and and are directly linked to the mesocosm construction later in Chapter 3. They were designed to replicate the upflow characteristic of local wetlands. The first cell was constructed using three layers of wetland-soils from wetlands adjacent to WPAFB. Each layer is approximately 18 inches thick. The lower layer was mixed with wood chips to provide an initial nutrient source of organic carbon for the microorganisms in the soil. The top two layers were unaltered in cell 1. Chapelle (2001) showed that iron facilitates the mineralization of certain chlorinated solvents. Thus, the second cell includes a layer of iron-rich soil, placed for the purposes of studying the process of reductive dehalogenases. Traditional wetland plant species were planted in the top layer which includes the sedges of interest: *Carex comosa*, *Scirpus atrovirens*, and *Eleocharis erythropoda*. The vegetation also introduces oxygen into the root zone enabling limited aerobic reactions to occur. A cross-sectional diagram of the first cell is shown in Figure 2.

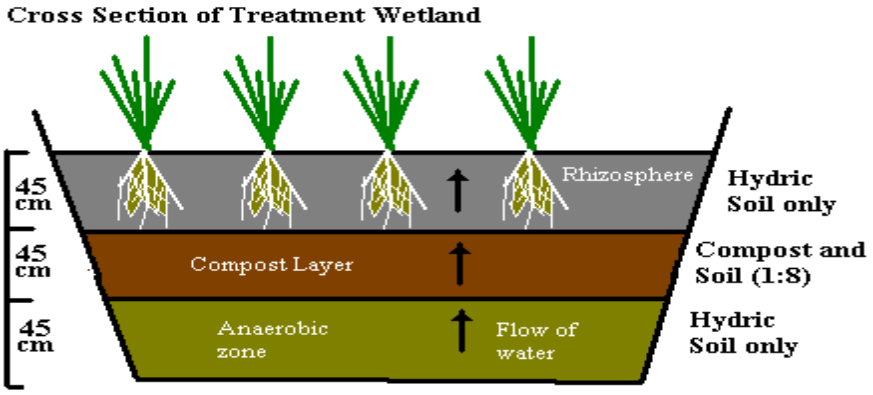


Figure 2. WPAFB Constructed Wetland Cross Section. (Enright, 2001)

Wetland Plants

The vascular plants associated with this study include a sedge (*Carex comosa*), a bulrush (*Scirpus atrovirens*), and a spike-rush (*Eleocharis erythropoda*), which are obligate wetland plant species from the sedge family, Cyperaceae (Reed, P. B., Jr., 1997) dominating regional wetlands (Amon, Thompson, Carpenter, & Miner, 2002). They are emergent monocots found across North America and dominate local wetlands. These plants possess, like many wetland plants, important adaptations to flooding and modify soil texture, hydraulic conductivity, and chemistry by growth of roots and rhizomes. One such adaptation is the development of aerenchymous tissue, which facilitates the transport of oxygen through vascular tissue from the atmosphere to roots, providing an aerated root zone (Kadlec & Knight, 1996). Lenticels are small stomata-like openings in plants tissue that allow for gas exchange into aeranchymous tissue network. This oxygen transport mechanism may be sufficient to provide for root metabolism only or may release excess oxygen to surrounding microbial populations. Gas-filled aerenchyma provide significantly less diffusional resistance, allowing oxidation of soils in the vicinity of the rhizosphere and diffusion of carbon dioxide, hydrogen sulfide, and even methane into the atmosphere (Kadlec & Knight, 1996).

Appropriate water levels and hydroperiods, duration of flooding or saturated soil conditions, are particularly important factors that determine wetland type and species dominance. Typical hydroperiod tolerances for the hydrophytes of interest are listed in Table 4.

Table 4. Hydroperiod Tolerance Ranges (Kadlec & Knight, 1996)

Species	Common Name	Maximum Water Depth (m)	Flooding Duration (Annual %)
Carex spp.	Sedges	<0.05-0.25	50-100
Eleocharis spp.	Spikerushes	<0.05-0.50	50-100
Scirpus spp.	Bulrushes	0.1-1.5	75-100

Most of the visible structure of wetlands is provided by vascular plants; however, the non-visible aspects are where the majority of contaminant modification occurs via microbial action and physical transformation processes. Plants provide structure and nutrient input for the microbes that mediate contaminant transformation. They allow oxygen transport to otherwise anaerobic soil and biomass provides an important carbon source in microbial degradation. They also influence the microenvironment in which they inhabit; for example, shading may inhibit algae growth, which impacts subsequent oxygen levels.

The role of root exudates and their impact on soil microbial ecology should also be considered. Exudates provide an important carbon source, may influence microbial resistance to pests, support symbiosis, or provide appropriate chemical composition (Bertin, Yang, & Weston, 2003) allowing certain microbes to inhabit niches, which in turn can provide for targeted remediation. Of course, different plant species affect soil chemistry in various ways. For example, de Ridder-Duine et al. (2005) revealed that the rhizosphere microbial community was mainly determined by bulk soil community for *Carex arenaria*.

Microbial Dechlorination

Dechlorination within a wetland is best understood by examining the biogeochemical processes that occur prior to reduction. Microorganisms first hydrolyze organic material producing organic monomers such as sugars, amino acids, and organic acids. Through fermentation microbes then form low-molecular weight acids, alcohols, and carbon dioxide from these monomers, which can be utilized by yet other microbes as energy or carbon sources (Chapelle, 2001). Microbial populations then use the alcohols and organic acids as electron donors in the production of acetate, formate, lactate, and molecular hydrogen. Sulfate-reducing microbes use these substrates as electron donors for metabolism. At low sulfate concentrations, iron-reducing microorganisms use acetate and hydrogen as electron donors. Methanogens and

halorespiring bacteria have also been shown to compete with sulfate- and iron-reducers at low iron concentrations (Chapelle, 2001).

Additionally, Dryzyzga et al. showed that syntropic relationships exist between sulfate-reducing and dehalorespiring bacteria at limited sulfate concentrations. This is accomplished through interspecies hydrogen transfer where the sulfate reducer gains energy by fermenting lactate and using dehalogenating bacteria as a biological electron acceptor. The sulfate-reducers respond by releasing hydrogen used in dehalorespiration (Drzyzga, Gerritse, Dijk, Elissen, & Gottschal, 2001).

Heterogeneity allows for mixtures of oxidation and reductive processes to occur spatially or temporally resulting in branched biodegradation pathways and complete degradation. The presence of reduced forms of PCE such as cis-DCE and VC in environments where no contamination of these chlorinated aliphatic hydrocarbons (CAHs) has occurred serves as evidence of microbial dehalogenation.

Hydrocarbons are removed from wetlands via five major routes: (1) volatilization, (2) photochemical oxidation, (3) sedimentation, (4) sorption, and (5) biological degradation (Kadlec & Knight, 1996). The main focus of this study relates to the biological pathway associated with the dechlorination of PCE. Chlorinated aliphatic hydrocarbon biodegradation occurs by five basic mechanisms: (1) abiotic dechlorination, (2) energy-yielding oxidation, (3) cometabolic oxidation, (4) cometabolic reductive dehalogenation, and (5) energy-yielding reductive dehalogenation (Lee, Odom, & Buchanan, 1998; Maier, Pepper, & Gerba, 2000). See Appendix B for a listing of known dechlorinating bacteria that use any of the pathways described below.

Abiotic Dehalogenation

Abiotic dehalogenation, also referred to as substitution, (see Figure 3) is a process in which the chlorine is substituted by the reaction of various complexes of reduced metals and

humic acids. Halogenated aliphatic compounds generally degrade slower than aliphatics without halogen substitution. Janssen et al.'s study (as cited in Maier, Pepper, & Gerba, 2000) shows that the presence of two or three chlorines bound to a carbon atom inhibits aerobic degradation. Bouwer (1994) observed that the abiotic dechlorination of PCE was approximately 6,000 times slower than reductive cometabolism.

$$CH_2{=}CHCl + H_2O \rightarrow CH_2CH_2OH + H^+ + Cl^-$$

Figure 3. Abiotic Substitution of VC.

Energy-Yielding Oxidation

Energy-yielding oxidation is known to occur in the aerobic degradation of reduced organochlorine species such as VC and DCE. Bacteria capable of using reduced chloroethenes as carbon sources include *Mycobacterium* sp., *Rhodococcus* sp., *Actinomycetales* sp., *Nitrosomonas* sp., *Nocardioides* strains, and possibly *Geobacter* sp. as cited by Coleman et al., (2002) and Lee et al., (1998).

Cometabolic Oxidations

Cometabolism occurs when a microbially produced enzyme degrades a substrate that is not used as a carbon source or for energy metabolism. The cometabolic degradation of a CAH may even be harmful to the microorganism responsible for the production of the enzyme or cofactor (Wiedemeier, Swanson, Moutoux, Wilson, Kampbell, Hanson, & Haas, 1997).

Methanotrophic bacteria produce the enzymes methane monooxygenase (MMO) and dioxygenase which act as catalysts for the oxidation reaction of methane to methanol. The enzymes are also known to oxidize chlorinated compounds. TCE, cis-DCE, and VC can be degraded in this manner via methanotrophic bacteria during the normal oxidation of hydrocarbons such as toluene, phenol, methane, or propane (Lee, Odom, & Buchanan, 1998; Chapelle, 2001). The presence of methane, however, competes for the available MMO,

hindering the degradation of chlorinated solvents (Semprini, 1995). This process has been used to degrade solvents when sufficient oxygen and co-substrates are present. PCE, however, has not been shown to degrade in this manner. Additionally, since it is rare for significant concentrations of methane to exist with dissolved oxygen, cometabolic oxidation is rare in most ground-waters and may require the addition of a substrate in order to produce effective enzyme expression (Chapelle, 2001).

Cometabolic Reductive Dehalogenation

Biodegradation of PCE within wetlands is thought to occur mainly by cometabolic reductive dehalogenation, since groundwater and hydrophilic soils are limited in oxygen. In these strictly anaerobic environments containing organic electron donors or hydrogen, this is thought to be the predominant mechanism of PCE dechlorination. Reductive dehalogenation is mediated by reduced transition metal complexes. In the first step of reductive dehalogenation, electrons are transferred from a reduced metal to the halogenated aliphatic, resulting in freeing of a halogen ion (Maier, Pepper, & Gerba, 2000). The reaction carried out by this type of bacteria is not considered energy-yielding but rather cometabolic because only a small fraction of the energy derived from the oxidation of electron donors is used to reduce the solvent. In wetlands, where high levels of organics and intense methanogenic or sulfidogenic respiration can be found, reductive dehalogenation can be significant (Lee, Odom, & Buchanan, 1998).

Energy-Yielding Reductive Dehalogenation: Dehalorespiration

Dehalorepiration refers to energy-yielding reductions where cells use the solvents as an electron acceptor for ATP-generation under anaerobic conditions. These bacteria differ from the cometabolic anaerobes found among sulfate-reducers and methanogens (Lee, Odom, & Buchanan, 1998). *Dehalococcoides ethenogenes* is the only species currently known to

completely dechlorinate PCE to ethene in this manner. Figure 4 provides an illustration of electron exchanges leading to the replacement of chlorine ions with hydrogen.

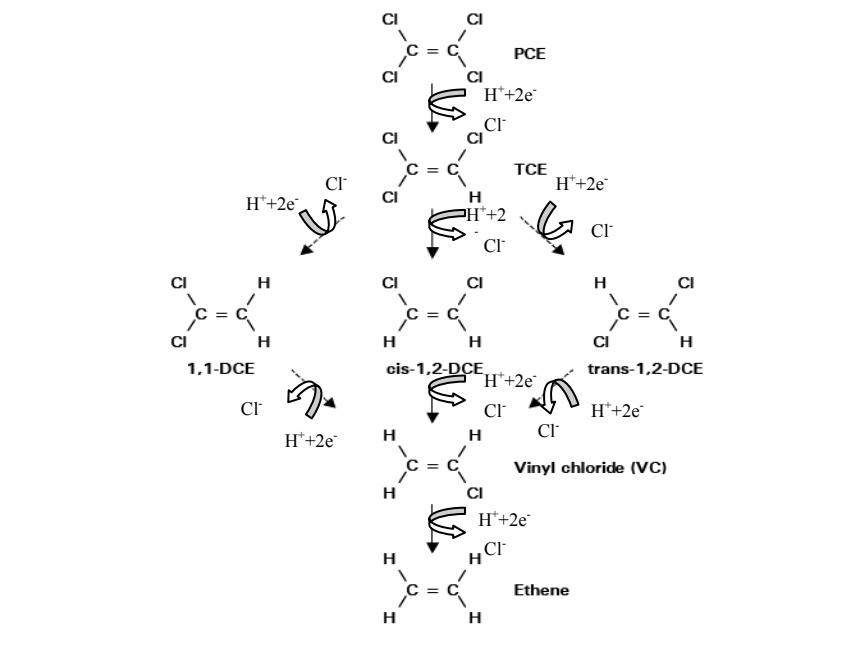


Figure 4. Reductive Dechlorination of PCE. (Adapted from Hageman, Istok, Field, Buscheck, & Semprini, 2001)

Aerobic conditions tend to favor biodegradation of compounds with few halogen substituents, while anaerobic conditions favor higher number halogen substituents. Therefore, complete degradation of PCE to ethene is typically dependant on mixed aerobic and anaerobic conditions. The four biodegradation pathways described above have been well studied and proven to provide the necessary reactions and conditions to remove and/or transform chlorinated solvents from groundwater. The sequential process is shown in Figure 5. In aerobic conditions, DCE and VC can be oxidized directly to carbon dioxide and chloride. At the interface between aerobic and anaerobic microenvironments, where methane and oxygen are both available, cometabolic oxidations can convert chlorinated ethenes to carbon dioxide and chloride. In anaerobic environments where electron donors such as organic carbon or hydrogen are present, reductive dehalogenation is the predominant mechanism (Lee, Odom, & Buchanan, 1998).

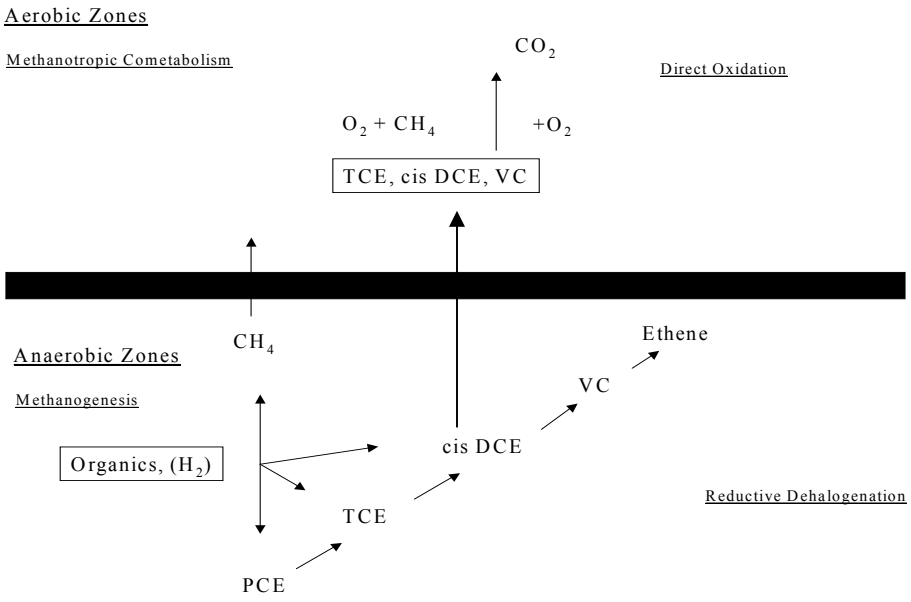


Figure 5. Relationship between different biological mechanisms within a wetland aquifer with both aerobic and anaerobic conditions (Lee, Odom, & Buchanan, 1998).

Microbial dechlorination of PCE occurs sequentially as described in Figures 4 and 5. PCE is first reduced to TCE and then to DCE, primarily *cis*-1,2-DCE (trans-1,2-DCE and 1,1-DCE are also possible isomer products, but are observed at low concentrations; Song, Conrad, Sorenson, & Alvarez-Cohen, 2002) then to VC, and ultimately, ethene. At each step a chloride ion (Cl^-) is replaced by a hydrogen ion (H^+) and two donated electrons. Reductive dechlorination of TCE occurs under Fe(III)-reducing, sulfate-reducing, and methanogenic conditions (Chapelle, 2001). Complete dechlorination of PCE has been observed under methanogenic conditions in the case of *Dehalococcoides ethenogenes* strain 195; however, the entire process is rarely completed by a single bacterium using the chlorinated ethene as an energy source or via cometabolism. More common is the incomplete degradation of PCE, and possible accumulation of its more harmful constituents.

Bacteria responsible for dechlorination belong to five different groups, including facultative anaerobes, nitrate reducers, and sulfate reducers (Holliger, Hahn, Harmsen, Ludwig, Schumacher, & Tindall, 1998).

DNA Analysis and Microbial Identification

Current estimates indicate that less than 0.5% of the microorganisms present in soil are readily culturable (Torsvik, Goksoyr, & Daae, 1990). The identification and assessment of microbial diversity and activity have been limited to the study of culturable microorganisms until the advent of advanced genetic techniques of detection.

Molecular techniques, such as separation of cells from soil followed by lysis of the cells to release the DNA, have become prevalent in order to conduct more precise assessments of the microbial community. They allow investigation of a community without culture biases by targeting ribosomal DNA (rDNA) for identification and providing a sequence to compare against all identified species. Specifically, sequence variation in the 16S rDNA gene has allowed for inferring evolutionary relatedness among microbes and is used to determine genetic diversity (Woese, 1987). The 16S rDNA gene has a length of approximately 1550 base pairs (bp) and provides sufficient information for phylogenetic analysis (S. A. Smith, 2005; Amann, Ludwig, & Schleifer, 1995). The primary steps of 16S rDNA analysis include (1) DNA extraction, (2) PCR amplification, (3) cloning, (4) sequencing, and (5) comparative analysis of retrieved sequences (Figure 6). This is a vast improvement over phenotypic methods, which only recover the culturable bacteria and offer little towards identifying bacterial dominance within a population. Thus, molecular techniques have greatly enhanced the understanding of microbial phylogeny and community composition.

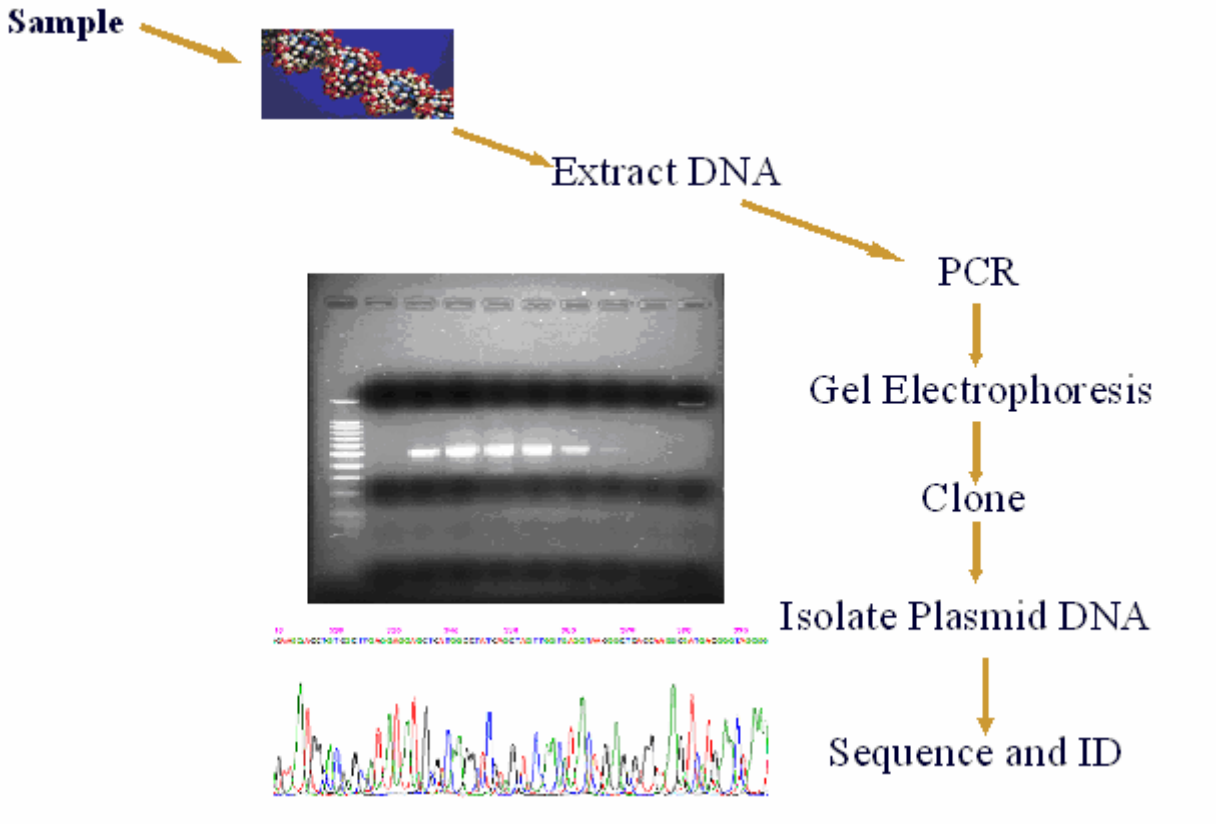


Figure 6. Steps in DNA analysis using PCR amplification.

Central to the tremendous increase in microbial identification and knowledge over the past 15 years has been the process of polymerase chain reaction (PCR) for obtaining sequences from environmental samples. This is an enzymatic reaction that allows amplification of DNA *in vitro*. A detailed description of PCR can be reviewed in Appendix D.

The use of PCR and its utility in providing an accurate characterization of the bacterial community, speed, sensitivity, and relatively low cost far out-weigh any disadvantages, while providing tremendous benefits, above and beyond traditional culture methods. Additionally, numerous computer programs assist in identifying and rectifying potential errors generated during this process.

DNA Extraction

Prior to PCR, the soil sample must be extensively processed to remove inhibitory substances and release DNA, a process known as extraction. Nucleic acids extracted from soil samples contain many impurities, such as humic acid and clay. Humic compounds are major inhibitors of PCR and interfere with lytic enzymes, bind to DNA and proteins, and interfere with DNA polymerase binding (Tsai & Rochelle, 2001). Thus, numerous extraction techniques have been developed in order to provide an extract which is as free as possible from humics. Optimal DNA extraction from soil samples with high humic content can be achieved by the glass bead beater method, using marketed kits such as the PowerSoil™ DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA, 2004), as described in the Methods section of this thesis and Appendix C.

Use of the 16S rRNA gene for Microbial Classification

The 16S rRNA gene sequence is about 1,550 bp long and is composed of both variable and conserved regions (Baker, Smith, & Cowan, 2003; Clarridge, 2004). The gene is large enough to provide distinguishing and statistically valid measurements of evolutionary relatedness and, thus, phylogentic placement. Through the sequencing of PCR-amplified 16S rDNA evolutionary similarity can be inferred and has revolutionized taxonomy. For example, differences among the three domains (Bacteria, Archaea, and Eucarya) was clarified by Carl Woese through the use of 16S rRNA sequence data and has been used to reconstruct the universal phylogeny of cellular life (Woese & Fox, 1977; Woese, 1987).

Choosing the correct primers for amplification of the 16S rDNA genes is dependent upon the research criteria. For example, if a specific genus of bacteria is being sought then only sequences within the variable regions that are unique to those bacteria are needed. However, for this study, where we wanted to identify as many members of a consortium as possible, so

sequences of the gene were utilized for primer annealing. The conserved sequences are sequences found in almost all known bacterial species and are considered "universal". Universal primers are complementary to the conserved regions within the 16S sequence, and the conserved regions are interspersed with variable regions that can be used for comparative taxonomy (Clarridge, 2004). However, research has shown that "universal" primers are not necessarily complementary to all sequences that exist in GenBank today, as discussed below (Baker, Smith, & Cowan, 2003).

Comparative Analysis & Phylogeny

Phylogenetic comparisons are made by comparison with listed sequences in databases such as GenBank (http://www.ncbi.nih.gov/Genbank), Ribosomal Database Project-II (http://rdp.cme.msu.edu), and European Molecular Biology Laboratory (http://www.ebi.ac.uk/embl/). GenBank is the largest databank of nucleotide sequences and has over 54 million deposited sequences, of which over 165,000 are from the 16S rRNA gene (Benson, Karsch-Mizrachi, Lipman, Ostell, & Wheeler, 2005). The Ribosomal Database Project-II database has over 101,632 bacterial small subunit rRNA gene sequences and assists in phylogenetically classifying isolated sequences (Cole, Chai, Farris, Wang, Kulam, & McGarrell, 2005).

Rochelle (2001) reports that "while many of the sequences display similarity to cultured and identified bacteria, each separate investigation generates 'novel' sequences with very little homology to recognized bacterial species." Some reportedly "novel" 16S rRNA sequences in GenBank contain large regions of cloning vector, possibly due to less than careful editing and analysis prior to submittal (Rochelle, 2001).

Tools for Sequence Analysis

BLAST (Basic Local Alignment Search Tool) is probably the best-known sequence analysis program, which compares two sequences through an algorithmic alignment process. The algorithm starts by looking for exact matches, and then expands the aligned regions by allowing for mismatches (Altschul, Gish, Miller, Myers, & Lipman, 1990). “Megablast is specifically designed to efficiently find long alignments between very similar sequences” (National Center for Biotechnology Information, 2006) and provides data such as percent identity to “hit”, e-value, and bit scores.

The expected value (E-Value) is the probability that you would observe a “hit” purely by chance when you do a search against a database of a particular size. The lower the E-value, the more "significant" the match is. However, the E-value calculation also takes into account the length of the sequence. Thus, shorter sequences have a higher probability of occurring in the database purely by chance (National Center for Biotechnology Information, 2006).

The “Bit Score” also represents a probability level for sequence comparisons that is independent of the size of the sequence. It serves an indication of how good the alignment is; the higher the score, the better the alignment.

By normalizing a raw score using the formula:

$$S' = \frac{\lambda S - \ln K}{\ln 2}$$

a "bit score" S' is attained, which has a standard set of units, and where K and lambda are the statistical parameters of the scoring system. A key element in this calculation is the BLOSUM62 substitution matrix, which assigns a score for aligning any possible

pair of residues and is well beyond the scope of literature review (National Center for Biotechnology Information, 2006).

There are many possible problems with the sequences produced which must be addressed prior to classification. They include residual removal outside primer sequences, gaps, ambiguous sequences ("N"), and nucleotide runs. Residuals can be easily removed using programs such as Bioedit v7.0.5 (Brown, 1999) software package or even Microsoft Word. These residuals are excluded when pairwise alignment is conducted in MegaBLAST which results in a match of the query against it closest matches or by trimming based on primer match. Sequences may also match a sequence's reverse compliment, which is easily identified in MegaBLAST.

A gap occurs when the sequencer either erroneously inserts a base or removes a base, causing a shift in alignment. When gaps are found the chromatogram should be viewed to determine possible causes. The chromatogram at this position may show that a base pair is missing or one has been added such as the addition of an "N" (Pruden, 2005). The matching base pair can be added or deleted.

Another editing step, which is should be done conservatively, is editing for Ns. For this there is a need to look at numerous BLAST pairwise alignments to the sequence submitted and determine if at the position of "N" there is consistent with a certain nucleotide. If there is consistency than it may be possible to change the "N" if the chromatogram shows the peak of that nucleotide is highest at that position. (Pruden, 2005)

Garrity, et al. (2004) present a classification scheme for prokaryotes based on 16S rRNA sequence analysis in *Bergey's Manual of Systematic Bacteriology* (Garrity, Bell, & Lilburn, 2004). RDP's Classifier program places the sequences into a taxonomic level consistent with

this updated taxonomy (Cole, Chai, Farris, Wang, Kulam, & McGarrell, 2005) and returns a value "based on the number of times, out of 100 trials, that random subsets of the query sequence match sequences assigned to that taxon;" (Janssen, 2006).

Phylogenetic trees are used to describe evolutionary relatedness between sequences. In order to show sequence relationships on a rooted tree, the most distant sequence is used to root the tree. The root describes common ancestory. Unrooted trees may also be used to describe evolutionary relatedness without identifying a common ancestor. As an example, if two sequences are very similar then they will be located adjacent to one another on outside branches (National Center for Biotechnology Information, 2004).

Species' rDNA sequence variations differ with respect to increasing phylogenetic distance. Comparisons are commonly shown as phylograms which show evolutionary relatedness of sequences against an "outgroup" the primary sequence against which a sequence is compared (Clarridge, 2004).

Taxonomic classification is a science in itself and is beyond the scope of this literature review. The literature offers a multitude of software programs and mathematical algorithms which enable a researcher to show evolutionary relatedness, however, no recognizably "correct" way to organize a tree is offered by taxonomists. Thus, it is left to the researcher to pick a method which enables a relatively clear representation of relatedness. Several web-based programs are available for estimating phylogenies and creating trees. PHYLIP (*PHYL*ogeny *I*nference *P*ackage) (Felsenstein, 2005) is one of these that is most commonly used.

Classification Considerations

Numerous problems exist when attempting to classify bacterial diversity, most notably the lack of taxonomic knowledge. It is difficult to describe diversity when there is no solid consensus on the proper way to categorize or identify species. The recognized definition of

species is based upon chromosomal DNA similarity. "The phylogenetic definition of species generally would include strains with approximately 70% or greater DNA-DNA relatedness and a 5ºC or less $\Delta T_{m,}$" (Liu & Stahl, 2002). For lack of a better alternative, bacterial taxonomists agreed to define a species on the basis of a DNA-DNA similarity of more than 70% (Wayne, Brenner, Colwell, Grimont, Kandler, & Krichevsky, 1988).

DNA-DNA relatedness is determined through DNA-DNA hybridization analysis and should not be confused with homology of 16S rRNA gene sequences. Stackebrandt and Goebel (1994) report, "if the isolate shares less than 97% sequence similarity with the nearest phylogenetic neighbor, then DNA-DNA reassociation studies are unnecessary, because the latter values will range clearly below the 70% reassociation borderline value recommended for species definition."(Stackebrandt & Goebel, 1994)

Diversity Statistics

Since soils microbial communities are so heterogenous and many species rare, it is difficult to determine the number of species from even an extensive sampling effort. Thus a number of indices have been derived to estimate species richness, diversity, and dominance. Diversity indices are numerous and each has its own strengths and weaknesses. The Shannon-Wiener diversity index:

$$H = \sum_{i=1}^{s} (P_i)(\ln P_i)$$

where P_i is the proportion of total sample belonging to *i*th species, is one such measure that is commonly used to measure diversity. It essentially measures the degree of uncertainty associated with predicting the identity of a randomly picked individual, e.g., "high diversity means high uncertainty" (R. L. Smith & Smith, 2003). Another common index used is the Simpson's Index:

$$D = 1 - \sum_{i=1}^{s} \left(P_i\right)^2 P_i$$

which is, essentially, the probability of picking two organisms at random that are different species (Krebs, 1978). The problem with the two preceding indices is that they rely on the assumption that the total number of species is known.

Chao proposed an estimator for species richness that takes the form:

$S^*_{Chao1} = S_{obs} + (a^2/2b)$

where S_{obs} is the number of species observed in a sample, "a" is the number of species observed once and "b" is the number of species observed just twice (Chao, 1984; Colwell, 2005).

Another useful estimate of species richness is the Abundance-based Coverage Estimator (ACE) (Chao & Lee, 1992; Chazdon, Colwell, Denslow, & Guariguata, 1998). This estimate is the proportion of all individuals in rare species that are not singletons. Singletons are individuals that show only one occurrence:

$$S_{ace} = S_{abund} + \frac{S_{rare}}{C_{ace}} + \frac{F_1}{C_{ace}} \gamma^2_{ace}$$

where F_i is frequency of *i* occurrences, S_{abund} is the number of common species (≥ 10 individuals), S_{rare} is number of rare species (<10 individuals), and γ^2 is the coefficient of variation of the F_i's found by:

$$\gamma^2_{ace} = \max\left[\frac{S_{rare}}{C_{ace}} \frac{\sum_{i=1}^{10} i(i-1)F_i}{(N_{rare})(N_{rare}-1)} - 1, 0\right], \text{ where}$$

$C_{ace} = 1 - \frac{F_1}{N_{rare}}$ is the sample coverage estimate, and

$N_{rare} = \sum_{i=1}^{10} iF_i$ is the total number of individuals in rare species.

"***Note:*** The formula for ACE is undefined when all Rare species are Singletons ($F_1 = N_{rare}$, yielding $C = 0$). In this case, EstimateS (the program used to compute estimates) computes the bias-corrected form of Chao1 instead" (Colwell, 2005).

If sampling effort is different among data sets, then rarefaction allows the comparison of the number of species found in two regions and answers how many species would have been found in smaller data set if sampling effort was equal (Hurlbert, 1971).

EstimateS is a program available from http://viceroy.eeb.uconn.edu/EstimateS (Colwell, 2005) that computes ACE and Chao1 non-parametric species richness estimates as well as Shannon-Weiner and Simpson's Indices among others. It does this through computing a data set input by the user in the correct tab-delimited format and using both randomization and rarefaction. Complete explanations of the equations used is lengthy and beyond the scope of this thesis. Colwell (2005) should be referred to for a more in depth explanation and links to cited literature.

Summary

Complete reductive dechlorination of PCE has been observed more commonly in mixed cultures or in field studies rather than pure cultures (Flynn, Löffler, & Tiedje, 2000). Knowledge about the microbial communities inhabiting wetland soils occupied by different species would greatly enhance the understanding of nutrient flux and the proper mix of organisms to incorporate when constructing a wetland for the purposes of treating chlorinated ethenes. Microbial populations and nutrient availability are fundamental elements in this process.

Flynn, et al. (2000) have suggested that at least two populations are responsible for sequential dechlorination of tetrachloroethylene (PCE) to ethene. Thus, knowledge of the biodegradation pathways of PCE and associated bacterial communities are of great importance. Genetic techniques are the means of achieving a more complete understanding.

III. Methodology

Experimental Overview

Previous research describing the dehalogenating aspects of the treatment wetland constructed at WPAFB dealt mainly with characterizing the transformation of chlorinated solvents to their more reduced forms. This research focuses on the relationship between the established dominant vegetation within the wetland cells and the subsurface microbial community. We investigate the microbial community in the rhizospheres of these plants prior to their exposure to PCE, in order to establish baseline data for comparison with rhizosphere communities that have been exposed to PCE in future studies.

The effects of *Carex comosa, Scirpus atrovirens*, and *Eleocharis erythropoda* on the spatial dominance of soil bacteria within a constructed reductive dechlorination wetland was evaluated through the construction of greenhouse mesocosms fed with dilute amounts of PCE through a vertical up-flow design representative of a ground water fed wetland. DNA was extracted from soil samples taken at different vertical depths after the establishment of plants within the mesocosms prior to PCE injection. 16S rDNA PCR and sequence analysis was used to examine the microbial communities within the rhizospheres of these plants.

Mesocosm Design and Construction

Twelve 5-foot mesocosms were constructed of 6-in PVC pipe at a height representative of the depth of the WPAFB treatment wetland. Soil sampling ports were then placed every 9-inches along the vertical length of the columns and sealed with PVC cement. Additional sampling ports were also placed along the column to allow for gas chromatography analysis. These ports were established radially to adjacent ports at 30 degrees offset in order to minimize upward flow disruption of water within the columns. The bottom 6 inches was filled with gravel to allow for even distribution of inflow. The inflow port was fitted with a 1/4 inch barbed fitting.

Water levels were maintained by gravity through 3/8 inch holes drilled 2 inches below the top of each column. Figure 7 provides a diagram of mesocosm design and sampling ports.

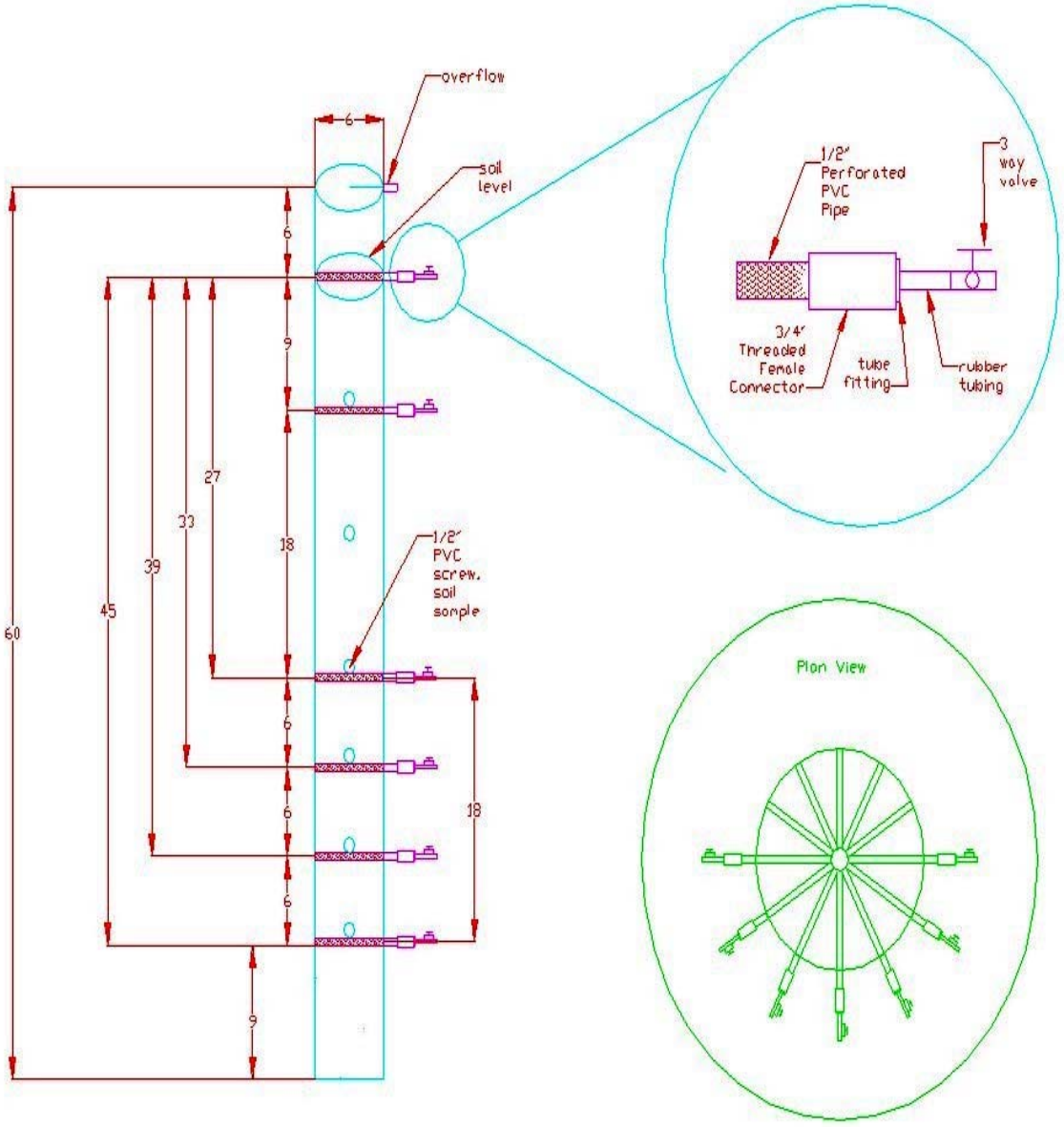


Figure 7. Column Design. All measurements are in inches. Soil sampling ports arranged every 9 inches.

Soil was obtained from the Beavercreek Wetlands and inoculated with soil from WPAFB treatment wetland. Inoculum was collected on 19 May 2005 by coring within the WPAFB

treatment wetland, Cell 1, followed by thorough homogenation in a mixing bed. Following soil homogenization columns were filled to a depth of 54 inches on 23 May 2005. Sedges were identified via dichotomous keys and planted with one species per column. The columns were arranged randomly (See Table 5), and the upflow conditions were replicated by pumping water into the columns at mean flow rate of 2.0 mL/min through Masterflex silicon tubing. Two peristaltic pumps fitted with 6 cartridges each pumped water from a 30 gallon water reservoir filled with distilled water and tap water at approximately a 1:1 ratio achieving a conductivity of 750 μS. PCE was injected beginning 7 Sept 2005 at a flow rate of 1.6 ml/hr between the peristaltic pumps and an intermediate mixing chamber in order to achieve an average PCE concentration of 50 ppb.

Table 5. Column Plantings

Column	Species
1	*Carex comosa*
2	*Carex comosa*
3	Blank
4	*Eleocharis erythropoda*
5	*Scirpus atrovirens*
6	*Scirpus atrovirens*
7	*Eleocharis erythropoda*
8	Blank
9	*Scirpus atrovirens*
10	*Eleocharis erythropoda*
11	Blank
12	*Scirpus atrovirens*

Soil Sampling

Two 50 g inoculated soil samples were taken at the time of homogenation and stored at -80°C in AFIT laboratory. On 23 August 2005, 5 g soil samples were aseptically taken from the center of each column through the sampling ports at depths of 13, 31, and 49 inches using a flamed metal spatula. Samples were collected with sterile 50 ml conical tubes. Significant root

mass was present in planted columns at shallowest depth while roots of *E. erythropoda* were encountered at mid-level samples.

Laboratory Methods

One of the greatest challenges in the PCR process is in maintaining unaltered/uncontaminated samples from the initial point of origin (mesocosms) through post-extraction procedures. Thus, great care was taken in ensuring sterile sampling tools and minimized transit time. Samples were taken directly from mesocosms at WSU greenhouse to an -80°C freezer within 45 minutes of extraction. Sterile conditions were maintained during DNA extraction and PCR by using a laminar flow hood, which was cleaned daily with 15% bleach and 70% ethanol, followed by a minimum of 15 minutes of UV light exposure. All equipment, glassware, and plasticware were autoclaved. Latex gloves were frequently changed and cleaned with bleach and ethanol dilutions. Glove exchanges occurred between samples in processes such as extraction, PCR preparation, and loading gel processes to prevent cross-contamination of samples.

DNA Extraction

Two 50 g samples of inoculated soil were obtained prior to addition of soils into mesocosms and labeled "SI5" and "SI6". After establishment of the emergent plants thirty-six 5 g samples were aseptically removed from the center of mesocosms through soil sampling ports at a depth of 13, 31, and 49 inches. Soil samples were labeled with the letter "A" signifying the month of extraction; 1st number represents column; 2nd number represents depth, with 1,2, and 3 representing bottom, middle, and top, respectively. (e.g. A=August, 1=Column 1; 2=Middle Depth).

Soil samples were centrifuged at 10,000 rpm for 20 seconds in order to eliminate excess water and allow for highest degree of soil wt/vol sample. Samples were then homogenized by

thoroughly mixing samples with plastic pestle for 30 seconds and 2 g subsamples extracted from each 5 g sample. Mo Bio's PowerSoil™ DNA Isolation Kit was used to purify and extract DNA from 0.25 g subsamples (Appendix C). Two buffer negative controls were included during each extraction evolution; these were soil negative samples ran simultaneously with the same protocol and solutions in order to ensure no contamination during extraction. For the controls, 250 µl of sterilized distilled water was used in place of the 0.25 g soil subsamples. DNA extracts were stored at -80°C.

PCR

PCR amplification was performed using a domain-specific 16S rRNA primer - E8F [5'AGAGTTTGATCCTGGCTCAG3', *E. coli* 16S rDNA positions 8 to 27] and the degenerate primer E533R [5'TIACCGIIICTICTGGCAC3', *E. coli* 16S rDNA positions 533 to 515], both with a final concentration of 0.4 µM. E533R uses deoxyinosine at nucleotide positions where PCR mismatches are common (Watanabe, Kodama, & Harayama, 2001). 16S rDNA was amplified from approximately 187 ng of extracted DNA (average concentration of template DNA was 17.8 ng/µl) prepared with PCR buffer, $MgCl_2$, deoxynucleoside triphosphates, primers, Qiagen's HotStarTaq polymerase (QIAGEN, Valencia, CA), and DNA template in 25 µl volumes following the protocol and dilutions outlined in Appendices D and F, respectively. An Eppendorf Mastercycler thermocycler with the program below was used:

- Lid Temp = 105°C
- Initial denaturation: T = 95°C for 15 minutes. Due to the unique attachment of an antibody preventing false denaturation, an extended denaturation time was necessary.
- Denaturation: T = 94°C for 1 minute.
- Annealing: T = 46°C (Empirically derived) for 1 minute.
- Extension: T = 72°C for 1 minute.
- 29 repetitions for a total of 30 cycles.
- Holding Temperature: T = 4°C.

Annealing temperatures (T_A) were estimated based on the size and composition of the primers as follows and confirmed by Baker et al. (2003):

E8F	E533R
$T_A = 4(G+C) + 2(A+T) - 5$ °C $= 4(6+4) +2(4+6) - 5$ °C $= 55$ °C	T_A empirically derived and proven effective from 40-55 °C (Watanabe et al., 2001).

*Note: Based on empirical testing the annealing temperature was lowered to 46 °C.

In order to minimize PCR bias in subsequent cloning steps, two separate reactions were run for each DNA extract. If reactions were successful, as determined by gel electrophoresis, they were pooled prior to subsequent cloning as described below.

DNA Concentrations

Unsuccessful gel electrophoresis results were common place, initially, due to changes in Taq polymerase used *(Qiagen's HotStarTaq* and *Promega's GoTaq*), high DNA concentrations present in template, or presence of inhibitory material. Other possible causes included problems in extraction resulting in residual humic material or contamination with Mo Bio's "C6" solution. To determine the root of negative results a spectrophotometer was used to determine DNA concentrations in gels with poor results. Spectrophotometer readings were obtained at a 1:100 concentration of template DNA to determine amount of DNA present prior to attempting a second PCR with those that gave false negative readings. Unfortunately, the lab's spectrophotometer was malfunctioning and DNA concentrations had to be derived empirically. Later readings on an ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE) revealed A_{260}/A_{280} and A_{260}/A_{230} ratios for the purified soil bacterium were 1.62 to 2.00 and 0.57 to 1.6, respectively, signifying relatively clean DNA extractions, although at widely varying concentrations.

Gel Electrophoresis

The PCR-amplified samples were loaded on a 0.8% agarose gel with 0.1% ethidium bromide and run at 95 V for 35 minutes at room temperature with a 1X TAE (Tris-Acetate-EDTA) buffer (see Appendix E for TAE recipe). The gel image was digitized using a Kodak Gel Logic 200 system. See Appendix G for results.

Cloning

The successful PCR amplifications were duplicated, pooled, then cloned using *Topo TA Cloning 2.1 Kit* (Invitrogen, Carlsbad, CA) and transformed into *Escherichia coli* according to protocol included in Appendix H. TOPO TA Cloning provides a highly efficient, one-step cloning strategy for the direct insertion of *Taq* polymerase-amplified PCR products into a plasmid vector (pCR2.1-TOPO®). The plasmid vector was supplied linearized with a single 3´-thymidine (T) overhang for TA Cloning and had the enzyme topoisomerase covalently bound to the vector. *Taq* polymerase used in PCR had a transferase activity that added a single adenosine (A) to the 3´ ends of PCR products allowing PCR inserts to ligate efficiently with the vector, while toposoimerase I binds to duplex DNA at specific sites and cleaves after 5′-CCCTT in one strand (Invitrogen Corporation, 2004).

The One Shot® *E. coli* competent cells were very fragile and were handled with care. They were stored at -80 °C and thawed on ice. Kanamycin at a concentration of 25 µg/ml was chosen as the selective agent in growth media. Three LB-kanamycin agar gel plates with 40 µg/ml X-gal were used per transformation with approximately 100 µl, 100 µl, and 80 µl, respectively, added to each plate. LB (Luria-Bertani) medium is a rich broth solution with a composition of 1% Tryptone, 0.5% yeast extract, 1.0% NaCl. The following recipe was used to prepare a 1 Liter solution:

Luria Bertani Medium (LB):

- 10.0g Tryptone (enzymatically digested milk protein casein - supplies amino acids)
- 5.0 g of Yeast Extract (supplies lots of nutrients)
- 1g glucose
- 10.0g NaCl
- deionized, distilled water to 1 liter
- Adjust to pH ~7.2

Plates were prepared by adding 15g/L agar, autoclaving on liquid cycle for 20 minutes at 15psi, cooled in tempering bath to 55°C, kanamycin (final concentration of 25 μg/ml) added, X-gal (final concentration of 40 μg/ml) added, and plates poured and allowed to cool.

After overnight incubation at 37°C, five white colonies were chosen from each plate and one blue colony (only for initial set of incubations in order to compare with white colonies) per transformation and grown overnight at 37°C in 2 ml LB-kanamycin (25 μg/ml) media. Qiagen's QIAprep Spin Miniprep Kit (Qiagen Inc., Valencia, CA) was used to purify and isolate plasmid DNA (Appendix L).

Restriction Enzyme Digestion

In order to test for successful insertion of PCR products into the Topo-TA vector, *Eco*R1 (Promega) restriction digestion followed by visualization on 1% agarose gel along with λ-HindIII DNA standard was done. The following is the digestion reaction mixture presented in the order prepared and steps:

- Sterile DI H2O---12.3 μl
- RE10X Buffer----2 μl
- BSA----------0.2 μl
- DNA----------5 μl
- Mix by pipetting
- *Eco*RI enzyme----0.5 μl
- Mix by pipetting
- Centrifuge ~30 sec @ 13,000 rpm
- Incubate at 37 °C for 3 hrs

Run entire sample on 1% agarose gel. See Appendix I for results.

Sequencing

Sequencing reactions were prepared using the GenomeLab Methods Development Kit (Beckman Colter Inc., Fullerton, CA). Dye terminator cycle sequencing reactions were prepared according to manufacturer's instructions with the M13 -47 sequencing primer and dITP sequencing chemistry (Appendix K). DNA template amounts were determined based on isolation concentrations attained from NanoDrop® ND-1000 spectrophotometer (Appendix J) and a recommended molar ratio of primer to template of ≥40:1(Beckman Coulter Inc., 2005). The DNA was also pre-heated for 1 min at 96 °C in order to ensure the plasmid was denatured, straightening it and releasing some of the impurities that adhere to it (Beckman Coulter Inc., 2005).

Samples were prepared and thermocycled on 96-well plates. Sequencing reactions were then transferred to polypropylene sample plates provided by Beckman-Coulter for use with the CEQ8000 sequencer. In these plates, samples were precipitated in 95% ethanol, and washed with 70% ethanol according to provided protocol. In order to remove remaining supernatant at the end of the ethanol wash, plates were centrifuged upside down at 200 rpm for 20 seconds, allowed to dry, and then pellets were resuspended in provided "Sample Loading Solution." Sequencing products were analyzed with a CEQ 8000 Genetic Analysis System (Beckman Colter Inc., Fullerton, CA).

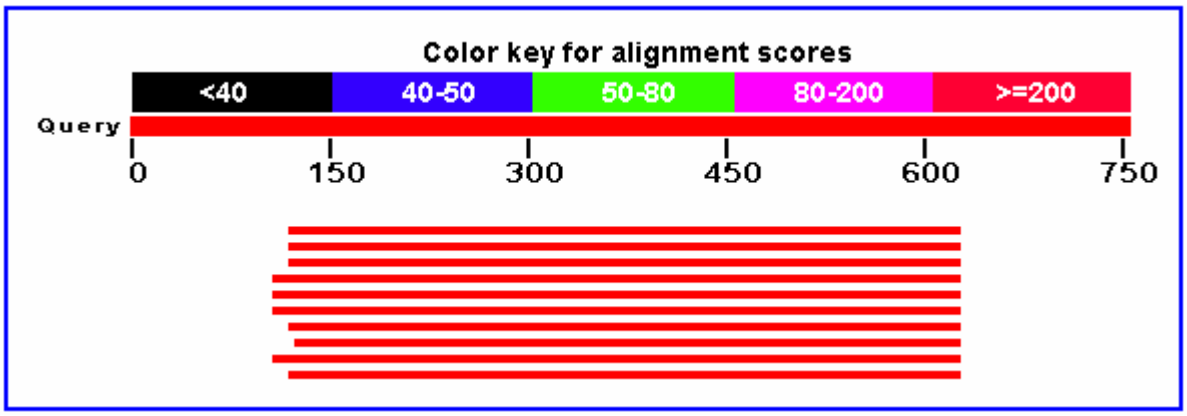
Comparative Analysis & Phylogeny

Nearest sequence matches were determined using NIH's MegaBLAST program (http://www.ncbi.nlm.nih.gov) (Altschul, Gish, Miller, Myers, & Lipman, 1990). The accession numbers ("AJ416168" as shown in Figure 8 below) are then cross referenced in order to provide a the closest match's entire sequence, definition, isolation source, and related research. The

ClustalW interface within Bioedit v7.0.5 (Brown, 1999) software package was used to align sequences to matching counterparts with the highest Bit Score.

Editing was done conservatively. Figure 8 is provided as an example of a BLAST result used for editing. The upper section of Figure 8 shows a summary of the sequence for matches against sample A12.2.3. The color red indicates a good match (>200 bp). Residuals (sequence segments outside of pairwise alignment) were removed using Bioedit v7.0.5 (Brown, 1999) software package after pairwise alignment was conducted in MegaBLAST. Pairwise alignment resulted in a matches of the "query" against it closest "subject." When gaps were found the chromatogram was viewed to determine possible causes. If the chromatogram at a gapped position showed that a base pair is missing or one has been added such as the addition of an "N" (Pruden, 2005) in a run of the same nucleotide, then the matching base pair was added or deleted.

Query= A12.2.3
Length=753



Alignments

```
>  gi|22265966|emb|AJ416168.1|ST416168  Uncultured bacterium partial 16S
rRNA gene, clone Sta0-45
Length=598

 Score =  798 bits (432),  Expect = 0.0
 Identities = 485/508 (95%), Gaps = 13/508 (2%)
 Strand=Plus/Plus

Query  121  GATGAAACGCTAGC-GGA-GCTTAACACATGCAAGTCGTGGGGCAGCACAGGTAGCAATA  178
            |||| ||||||||| ||| ||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GATG-AACGCTAGCGGGAGGCTTAACACATGCAAGTCGTGGGGCAGCACAGGTAGCAATA  59

Query  179  CTGGGTGGCGAACCGGCGCACGGGTGAGTAACGCGTATGCAACCTGCCCTGTACAGGGGG  238
            |||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  60   CTGGGTGGCGA-CCGGCGCACGGGTGAGTAACGCGTATGCAACCTGCCCTGTACAGGGGG  118

Query  239  ATAAGCCCGGAGAAATTCGGATTAATACCCCATAAAGATATGAGAAGGCATCTTTTTATA  298
            ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  119  ATA-GCCCGGAGAAATTCGGATTAATACCCCATAAAGATATGAGAAGGCATCTTTTTATA  177

Query  299  TTTAAAGTTTCGGCGGTACGGGATGGGCATGCGTGACATTATTTCTAGTTGGCAGGGTAA  358
            ||||||||||||||||||||||||||||||||||||||||   ||||||||||||||||
Sbjct  178  TTTAAAGTTTCGGCGGTACGGGATGGGCATGCGTGACATTAG--CTAGTTGGCAGGGTAA  235

Query  359  CGGCCTAACCAAGGCTTCGATGTCTAGGGGTCCTGAGAGGGTGATCCCCCACACTGGATA  418
            ||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct  236  CGGCCTA-CCAAGGCTTCGATGTCTAGGGGTCCTGAGAGGGTGATCCCCCACACTGG-TA  293

Query  419  CTGAGACACGGACCAGACTCCATACGGGAGGCATCAGTGAGGAATATTGGTCAATGGGCG  478
            |||||||||||||||||||| |||||||||| ||||||||||||||||||||||||||||
Sbjct  294  CTGAGACACGGACCAGACTCC-TACGGGAGGCAGCAGTGAGGAATATTGGTCAATGGGCG  352

Query  479  CAAGCCTGAACCCAACCCATCCCGCGTGCAAGAAGAACGCGCTATGCGTCGTAAACTGCT  538
            |||||||||||| | || |||||||||||| |||||| ||||||||||||||||||||||
Sbjct  353  CAAGCCTGAACC-AGCC-ATCCCGCGTGCAGGAAGAAGGCGCTATGCGTCGTAAACTGCT  410

Query  539  TTTGCAGGGGAAGAAAATCCCGTACGTGTACGGGACTGACCGTACCCTGTGAATAAGCAT  598
            ||| |||||||||||| |||||||||||||||||||||| ||||||||||||||||||||
Sbjct  411  TTTTCAGGGGAAGAAA-TCCCGTACGTGTACGGGACTGACGGTACCCTGTGAATAAGCAT  469

Query  599  CGGCTAACTCCGTGCCGGCAACCCCGGT  626
            |||||||||||||||| ||| || ||||
Sbjct  470  CGGCTAACTCCGTGCCAGCAGCCGCGGT  497
```

Figure 8. Pairwise BLAST alignment example.

Another editing step, which was done conservatively, is editing for "N" when it showed up in sequencing data. For this there was a need to look at numerous BLAST pairwise

alignments to the sequence submitted and determine if at the position of "N" there was consistency with a certain nucleotide. If there is consistency than it may be possible to change the "N" if the chromatogram shows the peak of that nucleotide is highest at that position. (Pruden, 2005)

Ribosomal Database Project-II's Classifier program (http://www.cme.msu.edu/RDP) was used to give an estimation of taxonomic placement based on an 80% confidence level. Edited sequences were used in this comparison.

Diversity Estimation

Relative abundance examinations were done on RDP-II Classifier results to relay any patterns noticed in phylotypes among plantings and depths. Richness estimates, rarefaction curves, and diversity indices were determined using EstimateS (http://viceroy.eeb.uconn.edu/EstimateS) for species level examination. Species richness was examined using the ACE and Chao1 estimates. Chao1 estimates of species richness were calculated after 1,000 randomizations of sampling without replacement. The percentage of coverage was calculated by Good's method with the formula [1-(n/N)] X 100, where n is the number of accession numbers represented by one clone (singletons) and N is the total number of sequences analyzed for the specified sampling (Good, 1953). Shannon-Weiner and Simpson's reciprocal indices were also determined despite an imperfect resolution of species abundance. EstimateS was utilized to calculate the preceding estimates of richness and indices incorporating rarefaction and randomization as outlined in Colwell (2005).

IV. Results & Discussion

PCR Results

The outcomes for all PCR reactions are provided in tables in Appendix F with corresponding agarose gels in Appendix G. Due to a malfunctioning spectrophotometer in the AFIT laboratory empirical testing of the appropriate volume of DNA template to use in PCR was necessary. DNA volumes ranged from 3 to 10.5 µl. Later testing revealed 16S rDNA was most successfully amplified from approximately 187 ng of extracted DNA. One contamination control blank resulted in a positive result (23 Sept Gel). However, when cloned and sequenced it matched closely with cloning vector sequences and sample A71NC (negative control – blue colony without insert). This indicates possible cross contamination from the pipet during one iteration of the PCR process.

Unsuccessful gel electrophoresis results were commonplace, initially, due to changes in Taq polymerase used *(Qiagen's HotStarTaq* and *Promega's GoTaq*), high DNA concentrations present in template, or presence of inhibitory material. Other possible causes included problems in extraction resulting in residual humic material or contamination with Mo Bio's "C6" solution. To determine the root of negative results a spectrophotometer was used to determine DNA concentrations in gels with poor results. Spectrophotometer readings were obtained at a 1:100 concentration of template DNA to determine amount of DNA present prior to attempting a second PCR with those that gave false negative readings. Unfortunately, the lab's spectrophotometer was malfunctioning and DNA concentrations had to be derived empirically. Later readings on an ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE) revealed A_{260}/A_{280} and A_{260}/A_{230} ratios for the purified soil bacterium were 1.62 to 2.00 and 0.57 to 1.6, respectively, signifying relatively clean DNA extractions, although at widely varying concentrations.

A total of 158 PCR amplifications were prepared in order to achieve duplicate positive results for the 36 mesocosm soil samples and 2 inoculated soil samples (38 total pools). Again, this number of amplifications was necessary due to the combination of changes in Taq polymerase used (which generated 28 negative results) and empirically derived concentrations necessary for effective amplification.

Cloning Results

No problems were encountered during transformation. Only one iteration of cloning using *Topo TA Cloning 2.1 Kit* (Invitrogen, Carlsbad, CA) and transformation into One Shot® *Escherichia coli* was necessary for all 38 PCR pools. The use of X-gal to visualize colonies with the plasmid insert allowed for efficient isolation of clones. Plasmid DNA purification proved problematic until it was realized that bacterial cell pellets were not properly resuspended in the first step with Buffer P1 using QIAprep Spin Miniprep Kit (Qiagen Inc., Valencia, CA) used to purify and isolate plasmid DNA. Thus, it became necessary to isolate a total of 746 clones in order to achieve plasmid DNA concentrations sufficient for sequencing a minimum of ten clones per original soil sample. After rectification of this problem, restriction digestion showed plasmid inserts for all samples (Appendix I). Clones were labeled according to original soil sample, plate number, and clone isolated from that plate. For example, in clone A10-3.2.5, "A" signifies the month of extraction; "10" represents the column number; "3" represents depth, with 1, 2, and 3 representing bottom, middle, and top, respectively; "2" is from first plate; and "5" is the fifth clone isolated.

Sequence Results

In the interest of brevity chromatograms were excluded from this thesis, however, all edited sequences can be viewed in Appendix M and an excerpt can be viewed in Figures 16 and 17 on pages 163 and 164, respectively. All results are maintained at sequencer workstation in

WSU microbiology laboratory. The results of the sampling and analytical procedures described in Chapter 3 are provided to present a detailed characterization of phylotype dominance in the various strata within each column. The results are intended to characterize the microbial community and provide an indication of possible relationships between wetland soils dominated by a particular species of plants.

Differences in identifications were not infrequent between BLAST and RDP-II sequence comparisons. The two programs acquire their sequences from the same databases; however, search results differ because BLAST searches against all available sequences, while RDP-II uses only select GenBank sequences within their own database as reference sequences. Additionally, the results may be partially inaccurate or misleading since GenBank and RDP-II are public databases constructed from non-peer reviewed submissions. It should also be remembered that BLAST results with the highest bit scores may also be a result of sequence alignment length, resulting in identification of a species with the highest score despite having significantly lower percentage similarity. Regardless, BLAST results proved consistent, reproducible, and statistically valid based on algorithms used to determine e-values. Thus it was used to provide nearest sequence matches which were in turn edited and used in RDP's Classifier program.

A total of 396 sequences were attained. Of these, 34 were excluded from the RDP classification: 4 from the positive contamination control blank, one from a blue colony ("A71.NC") which received no PCR insert, and 29 which were under 200 bp in alignment length. The average sequence length of these 362 clones was 692 bp with an average alignment length of 475 bp. The average percent identity, e-value, and bit score were 92.8%, 1.66E-44, and 680, respectively.

Phylogenetic analysis

Of the 362 clones carried on to the RDP Classifier, 99.4% were classified as belonging to the domain Bacteria under 11 separate phyla. Nine monophyletic phyla (consists of a common ancestor) were represented and two unique phyla (*Genera incertae sedis OP11* and *OP10*) showing unresolved common ancestory. Figure 9 displays RDP Classification results for domain and phyla classifications. As seen from Figure 9 and Table 6, the majority of sequences (50.8%) were unclassified, meaning that random subsets of the query sequence did not match sequences assigned to that taxon greater than or equal to 80% of the time. Yet, 32.8% of sequences are from the phylum *Proteobacteria*, which represents 66.67% of the 177 classifiable sequences.

domain	%	Library
Bacteria	99.4	
unclassified Root	0.6	

phylum	%	Library
Genera_incertae_sedis_OP11	0.3	
Genera_incertae_sedis_OP10	0.3	
Nitrospira	1.7	
Verrucomicrobia	1.1	
Gemmatimonadetes	0.6	
Bacteroidetes	3.1	
Chloroflexi	3.3	
Planctomycetes	0.8	
Actinobacteria	4.4	
Proteobacteria	32.8	
Firmicutes	0.8	
unclassified Bacteria	50.8	

Figure 9. RDP results for 362 clones with sequence alignment lengths >200 bp. 80% Confidence (Calculated by RDP's Classifer program). Detailed lineage profile can be viewed in Appendix O.

Phylum Level Diversity

Mid-level of columns showed highest abundance of phylum level richness, with 9 different phyla represented, compared to 7 and 8 phyla in levels 1 and 3, respectively. (Table 6), The *Proteobacteria* clearly dominated throughout all depths. Phylum *Chloroflexi* was prevalent at the lowest depth, where it comprised12.2% if the represented phyla compared to roughly 5% in levels 2 and 3. *Gemmatimonadetes* were only represented at the highest level (level 3).

Few remarkable conclusions can be made when comparing planting to phyla occurrence (Table 7) due to a small number of represented occurences. The control columns containing no plants supported the highest phylum level abundance. *Chloroflexi*, however, was more prevalent in the *S. atrovirens* mesocosms representing 9.8% of the classifiable clones compared to 3.6% and 2.8% in the *C. comosa* and *E. erythropoda*, respectively. *Actinobacteria* was also more prevalent in soil samples used as the original inoculum for the mesocosms, than in those samples later taken from planted mesocosms. While *Chloroflexi* occurred across all three depths, it showed greatest abundance at the lowest depth (Tables 8 and 9). The relative percentages of other represented phyla were, otherwise, relatively even across all three plantings.

Table 6. Phylum affiliation to depth of classified clones. n = the frequency of occurrence at 80% confidence; percentage represents number clones per total classified at 80% confidence under each depth.

Phylum	SI (Inoc. Soil)	Mesocosm Depth			All Classifiable Clones
		Level 1 (49 in)	Level 2 (31 in)	Level 3 (13 in)	
Genera incertae sedis OP11			1		1
			1.7%		0.6%
Genera incertae sedis OP10			1		1
			1.7%		0.6%
Nitrospira		2	2	2	6
		4.1%	3.4%	3.4%	3.4%
Verrucomicrobia		1	3		4
		2.0%	5.2%		2.3%
Gemmatimonadetes				2	2
				3.4%	1.1%
Bacteroidetes	1	5	3	2	11
	9.1%	10.2%	5.2%	3.4%	6.2%
Chloroflexi		6	3	3	12
		12.2%	5.2%	5.1%	6.8%
Planctomycetes	1		1	1	3
	9.1%		1.7%	1.7%	1.7%
Actinobacteria	2	3	6	5	16
	18.2%	6.1%	10.3%	8.5%	9.0%
Proteobacteria	7	31	37	43	118
	63.6%	63.3%	63.8%	72.9%	66.7%
Firmicutes		1	1	1	3
		2.0%	1.7%	1.7%	1.7%
Classifiable Bacteria	11	49	58	59	177
Unclassfied Bacteria	9	71	55	48	183
Total	20	120	113	107	360

Table 7. Phylum affiliation to plant species. n = the frequency of occurrence at 80% confidence; percentage represents number clones per total classified at 80% confidence under each planting.

Phylum	SI (Inoc. Soil)	Mesocosm Planting				All Classifiable Clones
		C. comosa	*E. erythropoda*	*S. atrovirens*	None	
Genera incertae sedis OP11					1 2.4%	1 0.6%
Genera incertae sedis OP10					1 2.4%	1 0.6%
Nitrospira		1 3.6%	1 2.8%	2 3.3%	2 4.9%	6 3.4%
Verrucomicrobia			2 5.6%	1 1.6%	1 2.4%	4 2.3%
Gemmatimonadetes		1 3.6%	1 2.8%			2 1.1%
Bacteroidetes	1 9.1%	2 7.1%	2 5.6%	5 8.2%	1 2.4%	11 6.2%
Chloroflexi		1 3.6%	1 2.8%	6 9.8%	4 9.8%	12 6.8%
Planctomycetes	1 9.1%			1 1.6%	1 2.4%	3 1.7%
Actinobacteria	2 18.2%	2 7.1%	3 8.3%	6 9.8%	3 7.3%	16 9.0%
Proteobacteria	7 63.6%	20 71.4%	25 69.4%	40 65.6%	26 63.4%	118 66.7%
Firmicutes		1 3.6%	1 2.8%		1 2.4%	3 1.7%
Classibiable Bacteria	11	28	36	61	41	177
Unclassfied Bacteria	9	21	58	53	42	183
Total	20	49	94	114	83	360

Genera Level Diversity

Twenty-two genera were represented with 80% confidence. It was somewhat surprising that 40 clones (or 11% of all edited sequences) could be classified to the Genus level using RDP's Classifier program, even though many of the accession numbers were defined as "uncultured bacteria" by BLAST. For example, sample A73.2.3 nearest match was with BLAST accession number DQ125856 with a percent identity of 97.27% and e-value of 0.0, an uncultured bacterium clone; yet it still showed a 100% similarity to the genus *Acetivibrio* in RDP. The genera *Anaerolinea* and *Nitrospira* from the phylum *Chloroflexi* and *Nitrospira*, respectively, proved highly prevalent at all levels, representing 30% and 15% of clones classifiable to genus level at 80% confidence (Table 8). Amongst the plantings (Table 9) the genus *Anaerolinea* proved prevalent amongst the *S. atrovirens* plantings. *Chloroflexi* (green non-sulfur bacteria) are described as facultatively aerobic organisms; however, *Anaerolinea thermophila* represents the

sole species of the genus *Anaerolinea* and was shown to be a strictly anaerobic organism (Sekiguchi, Yamada, Hanada, Ohashi, Harada, & Kamagata, 2003). Level 2 showed most even distribution of genera (Figure 10). *E. erythropoda* demonstrated most the most proportional distribution of genera among planted columns, while *S. atrovirens* displayed a higher degree of genera richness (Fig. 11), probably due to increased sampling as the result of 2 plantings of *C. comosa* and 4 plantings of *S. atrovirens*.

Table 8. Genus affiliation of classified clones. Percentage represents number clones per total classifiable to genus level (40) at 80% confidence.

Phylum	Class/Subclass	Genus	SI (Inoc. Soil)		Mesocosm Depth						Total	
					Level 1 (49 in)		Level 2 (31 in)		Level 3 (13 in)			
Actinobacteria	*Actinobacteridae*	*Microlunatus*	1	2.50%							1	2.50%
		Mycobacterium			1	2.50%					1	2.50%
		Nocardioides					1	2.50%			1	2.50%
	Rubrobacteridae	*Solirubrobacter*					1	2.50%			1	2.50%
Bacteroidetes	*Flavobacteria*	*Flavobacterium*					1	2.50%			1	2.50%
	Sphingobacteria	*Chitinophaga*					1	2.50%			1	2.50%
Chloroflexi	*Anaerolineae*	*Anaerolinea*			6	15.00%	3	7.50%	3	7.50%	12	30.00%
Firmicutes	*Clostridia*	*Acetivibrio*							1	2.50%	1	2.50%
Gemmatimonadetes	*Gemmatimonadetes*	*Gemmatimonas*							2	5.00%	2	5.00%
Genera incertae sedis OP10		*OP10*					1	2.50%			1	2.50%
Genera incertae sedis OP11		*OP11*					1	2.50%			1	2.50%
Nitrospira	*Nitrospira*	*Nitrospira*			2	5.00%	2	5.00%	2	5.00%	6	15.00%
Planctomycetes	*Planctomycetacia*	*Planctomyces*					1	2.50%			1	2.50%
Proteobacteria	*Alphaproteobacteria*	*Hyphomicrobium*							1	2.50%	1	2.50%
	Betaproteobacteria	*Hydrogenophaga*	1	2.50%							1	2.50%
		Nitrosospira							1	2.50%	1	2.50%
	Deltaproteobacteria	*Anaeromyxobacter*							2	5.00%	2	5.00%
		Desulforegula			1	2.50%					1	2.50%
		Geobacter					1	2.50%			1	2.50%
		Haliangium			1	2.50%					1	2.50%
		Pelobacter			1	2.50%					1	2.50%
Verrucomicrobia	*Verrucomicrobiae*	*Verrucomicrobium*			1	2.50%					1	2.50%
Total			2	5.00%	13	32.50%	13	32.50%	12	30.00%	40	100.00%

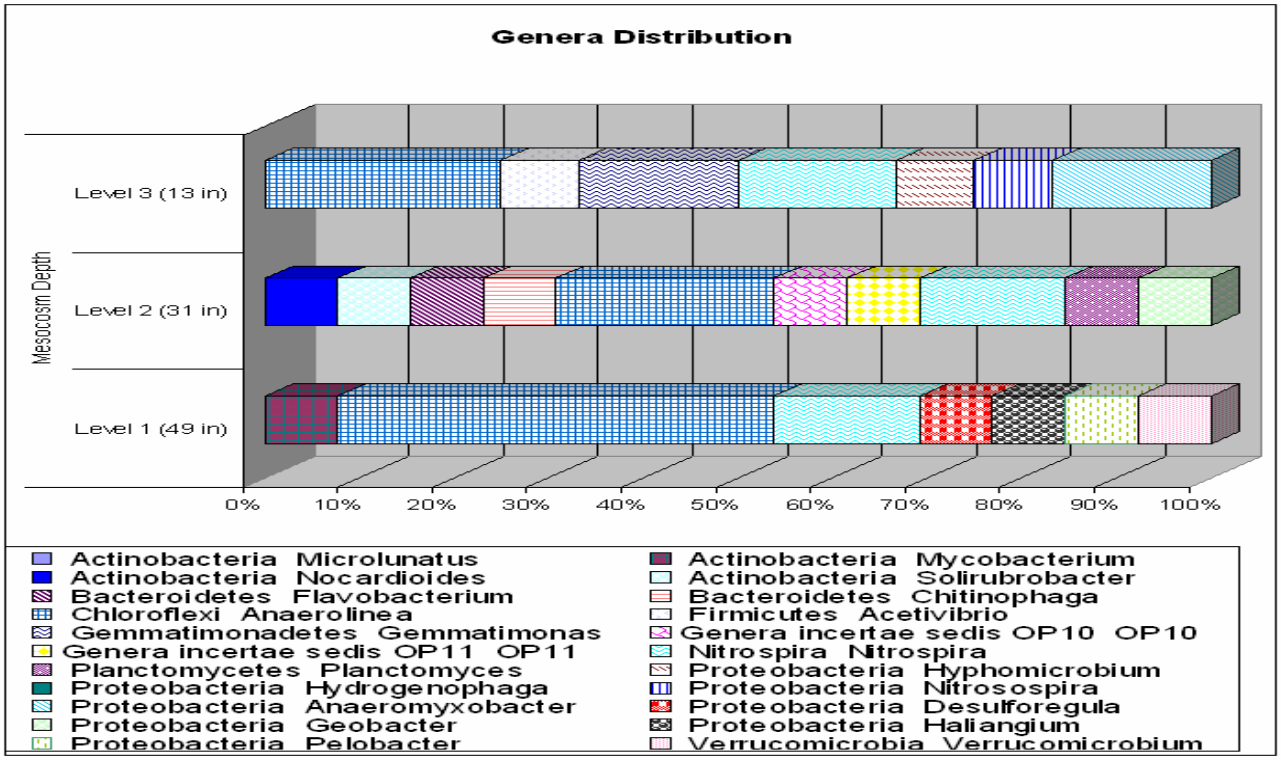


Figure 10. Genera Distribution Among Depths.

Table 9. Genus affiliation to plant species. n = the frequency of occurrence at 80% confidence; percentage represents number clones per total classifiable to genus level per plant species.

Phylum	Class/Subclass	Genus	SI (Inoc. Soil)		Mesocosm Depth								Total	
					C. comosa		*E. erythropoda*		*S. atrovirens*		None			
Actinobacteria	*Actinobacteridae*	*Microlunatus*	1	50.00%									1	2.50%
		Mycobacterium									1	9.09%	1	2.50%
		Nocardioides							1	6.67%			1	2.50%
	Rubrobacteridae	*Solirubrobacter*					1	14.29%					1	2.50%
Bacteroidetes	*Flavobacteria*	*Flavobacterium*									1	9.09%	1	2.50%
	Sphingobacteria	*Chitinophaga*							1	6.67%			1	2.50%
Chloroflexi	*Anaerolineae*	*Anaerolinea*			1	20.00%	1	14.29%	6	40.00%	4	36.36%	12	30.00%
Firmicutes	*Clostridia*	*Acetivibrio*					1	14.29%					1	2.50%
Gemmatimonadetes	*Gemmatimonadetes*	*Gemmatimonas*			1	20.00%	1	14.29%					2	5.00%
Genera incertae sedis OP10		*OP10*									1	9.09%	1	2.50%
Genera incertae sedis OP11		*OP11*									1	9.09%	1	2.50%
Nitrospira	*Nitrospira*	*Nitrospira*			1	20.00%	1	14.29%	2	13.33%	2	18.18%	6	15.00%
Planctomycetes	*Planctomycetacia*	*Planctomyces*							1	6.67%			1	2.50%
Proteobacteria	*Alphaproteobacteria*	*Hyphomicrobium*					1	14.29%					1	2.50%
	Betaproteobacteria	*Hydrogenophaga*	1	50.00%									1	2.50%
		Nitrosospira							1	6.67%			1	2.50%
	Deltaproteobacteria	*Anaeromyxobacter*			1	20.00%			1	6.67%			2	5.00%
		Desulforegula							1	6.67%			1	2.50%
		Geobacter					1	14.29%					1	2.50%
		Haliangium			1	20.00%							1	2.50%
		Pelobacter							1	6.67%			1	2.50%
Verrucomicrobia	*Verrucomicrobiae*	*Verrucomicrobium*									1	9.09%	1	2.50%
Total			2	100.00%	5	100.00%	7	100.00%	15	100.00%	11	100.00%	40	100.00%

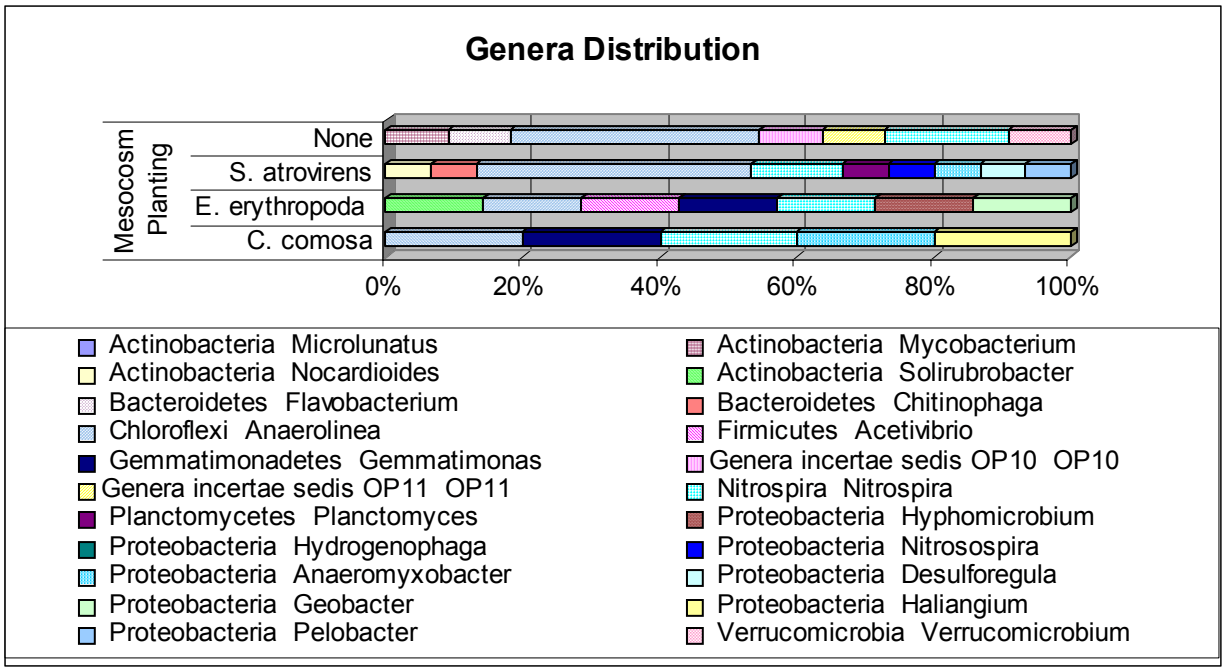


Figure 11. Genera distribution among plantings.

Species Diversity

Classifier proved useful in inferring phylogeny based on classified sequences. BLAST results, however, were used to determine species level diversity, since this database represents all known sequences and is the most heavily cited tool for such identifications.

For the species diversity indices, the 20 soil inoculum samples were excluded leaving 342 clones. There were 288 accession numbers represented by these 342 clones (Appendix Q). Tables 10-12 list duplicated accession numbers with number of repeats and their relation to depth/planting. These duplicates were represented in the data inputs to EstimateS, which was used to calculate diversity incices as described in the Methodology section. Due to increased sampling effort involving *S. atrovirens* many of the duplicates came from the 12 samples extracted from these mesocosms, while *C. comosa*, present in only two mesocosms, showed only two repeats.

Table 10. Duplicated accession numbers.

Accession #	# Duplicates	Accession #	# Duplicates	Accession #	# Duplicates
AB234243	3	AM085466	2	DQ154336	2
AB240237	2	AY102911	2	DQ154346	2
AB240264	2	AY188292	3	DQ154377	2
AB240280	2	AY212696	2	DQ154435	3
AF293010	2	AY214798	2	DQ154525	7
AF392740	2	AY360642	2	DQ154551	3
AF418945	2	AY568858	5	DQ154600	2
AF419683	2	AY921821	4	DQ154627	2
AJ232797	3	AY921932	2	DQ154633	2
AJ306790	2	DQ065033	5	DQ154649	2
AJ518795	2	DQ066684	4	DQ223206	2
AJ585959	2	DQ093937	2	DQ297986	2
AJ863223	2	DQ125726	2	DQ310755	2
AJ876729	2	DQ128428	2	Z95736	2

DQ154525 (uncultured soil bacterium) was the most prevalent sequence match and occurred among all plantings and at depths. RDP classified these sequences matching DQ154525 as *Deltaproteobacteria*.

Table 11. Depth and corresponding duplicates.

Mesocosm Depth											
Level 1 (49 in)				Level 2 (31 in)				Level 3 (13 in)			
Accession #	Definition	Planting	# Repeats	Accession #	Definition	Planting	# Repeats	Accession #	Definition	Planting	# Repeats
AF392740	Uncultured bacterium clone LAC1 16S ribosomal RNA gene	None	2	AM085466	Uncultured bacterium partial 16S rRNA gene, clone E173	None	2	AF419683	Uncultured bacterium CS_B017 16S ribosomal RNA gene, partial sequence	*S. atrovirens*	2
AJ306790	Uncultured bacterium partial 16S rRNA gene, clone SHA-59	*E. erythropoda*	2	AY214798	Uncultured Acidobacteria bacterium clone BB-2-H5 16S ribosomal RNA gene	*E. erythropoda*	2	AY188292	Uncultured bacterium clone KD1-11 16S ribosomal RNA gene, partial sequence	*C. comosa*	2
AY102911	Uncultured bacterium clone BCM3S-5B 16S ribosomal RNA gene, partial sequence	*S. atrovirens*	2	AY568858	Uncultured bacterium isolate JH12_C17 16S ribosomal RNA gene, partial sequence	*E. erythropoda*	2	DQ154435	Uncultured soil bacterium clone RFS-C109 16S ribosomal RNA gene, partial sequence	None	2
AY568858	Uncultured bacterium isolate JH12_C17 16S ribosomal RNA gene, partial sequence	*S. atrovirens*	2	AY921821	Uncultured beta proteobacterium clone AKYH490 16S ribosomal RNA gene	None	3	DQ154525	Uncultured soil bacterium clone RFS-C208 16S ribosomal RNA gene, partial sequence	None	2
DQ065033	Uncultured freshwater bacterium clone 965019A11.x1 16S ribosomal RNA gene, partial sequence	*S. atrovirens*	2	DQ065033	Uncultured freshwater bacterium clone 965019A11.x1 16S ribosomal RNA gene, partial sequence	*C. comosa*	2	DQ223206	Uncultured proteobacterium clone EV221H2111601SAH33 16S ribosomal RNA gene, partial sequence	*S. atrovirens*	2
DQ154525	Uncultured soil bacterium clone RFS-C208 16S ribosomal RNA gene, partial sequence	*C. comosa*	3	DQ154346	Uncultured soil bacterium clone RFS-C16 16S ribosomal RNA gene, partial sequence	None	2				
				DQ154525	Uncultured soil bacterium clone RFS-C208 16S ribosomal RNA gene, partial sequence	*E. erythropoda*	2				

Table 12. Plants and corresponding duplicates.

C. comosa		*E. erythropoda*		*S. atrovirens*		*None*	
Accession #	***# Repeats***	***Accession #***	***# Repeats***	***Accession #***	***# Repeats***	***Accession #***	***# Repeats***
DQ154525	3	AY214798	2	AB234243	2	AF392740	2
Z95736	2	AY568858	2	AB240264	2	AY188292	2
		DQ154525	2	AY102911	2	AY921821	2
				AY568858	3	DQ066684	2
				DQ065033	2	DQ154627	2
				DQ093937	2		
				DQ154633	2		
				DQ223206	2		

Since sampling effort differed due to a greater number of mesocosms being planted with *S. atrovirens* and alignment lengths under 200 bp were excluded, rarefaction (incorporated in EstimateS program) was used in the calculation of species richness and diversity indices for each plant type and depth. Rarefaction, as outlined in Chapter II, allows the comparison of the number of species found in two regions and answers how many species would have been found in a smaller data set if sampling effort was equal (Hurlbert, 1971).

Table 13. Diversity indices and richness estimates based on BLAST results. Note: Numbers given for plantings related to depth (and vice versa) were not included, since pooled sampling data was too small to allow for doubletons.

	Level 1	**Level 2**	**Level 3**	***C. comosa***	***E. erythropoda***	***S. atrovirens***	**None**
Individuals	122	113	107	49	96	114	83
Sobs	115	105	102	46	93	105	78
ACE Mean	1252.86	883.28	1091.4	680.77	1488	764.73	647.4
Chao 1 Mean	1096	784	878	519	1094.25	687	516
Shannon Index	4.72	4.62	4.61	3.8	4.52	4.62	4.34
Simpson's Reciprical Index	922.63	703.11	1134.2	294	1520	644.1	680.6
Good's Coverage	5.7%	7.1%	4.7%	6.1%	3.1%	7.9%	6.0%

$ShannonIndex = \sum_{i=1}^{s} (P_i)(\ln P_i)$; $Simpson'sIndex = 1 - \sum_{i=1}^{s} (P_i)^2 P_i$; $S^*_{Chao1} = S_{obs} + (a^2/2b)$; and

$S_{ace} = S_{abund} + \frac{S_{rare}}{C_{ace}} + \frac{F_1}{C_{ace}} \gamma^2_{ace}$

Table 13 and Figures 12-18 provide a good perspective of just how diverse the soil microbial community was. Although the mid-level soil proved to be more diverse at the phylum and genera level, lower species level diversity was shown for mid-level samples. This may

occur when fewer species are observed among a smaller range of higher level phylotypes or may be a result of a higher percentage of clones which were "unclassifiable," thus not included in Classifier based examination. The *E. erythropoda* columns showed much more species richness than the other columns; this may be due in part to the plant's roots providing an environment capable of supporting differing metabolic conditions.

C. comosa columns contributed 6 soil samples and 49 clones resulting in 46 observed species; *E. erythropoda* columns contributed 9 soil samples and 96 clones resulting in 93 observed species; *S. atrovirens* columns contributed 12 soil samples and 114 clones resulting in 105 observed species; and the columns with no planting contributed 9 soil samples and 83 clones resulting in 78 observed species. Levels 1, 2, and 3 contributed 12 soil samples each corresponding to 122, 113, and 107 clones and 115, 105, 102 observed species, repectively.

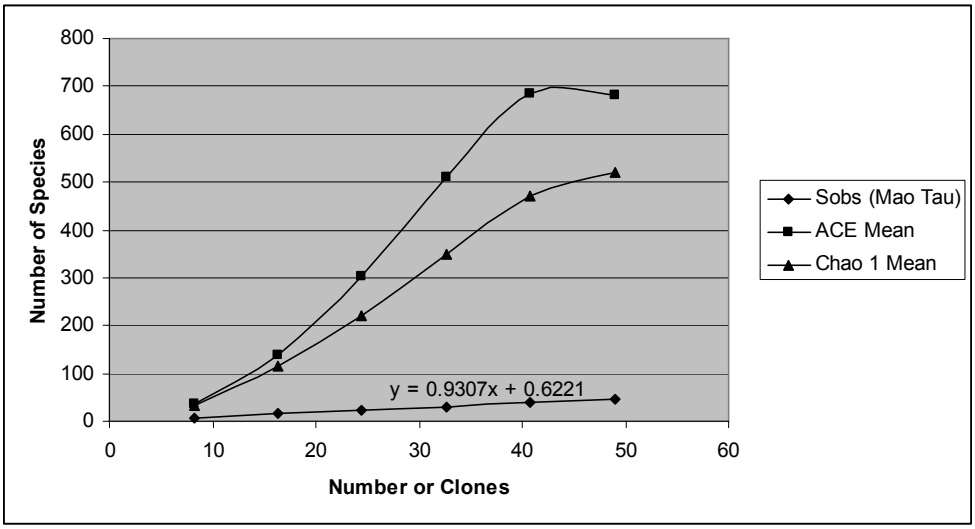


Figure 12. *C. comosa* rarefaction curve of number of observed species (S_{obs}) from 16S rRNA gene clone library recovered from mesocosms. Computed by EstimateS. The corrected ACE and Chao1 estimators (after 1,000 randomizations) were also plotted.

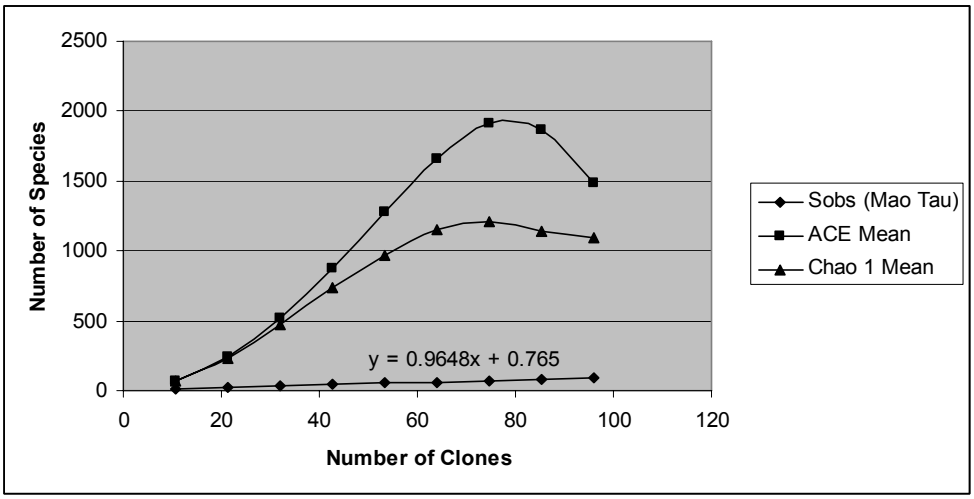


Figure 13. *E. erythropoda* rarefaction curve of number of observed species (S_{obs}) from 16S rRNA gene clone library recovered from mesocosms. Computed by EstimateS. The corrected ACE and Chao1 estimators (after 1,000 randomizations) were also plotted.

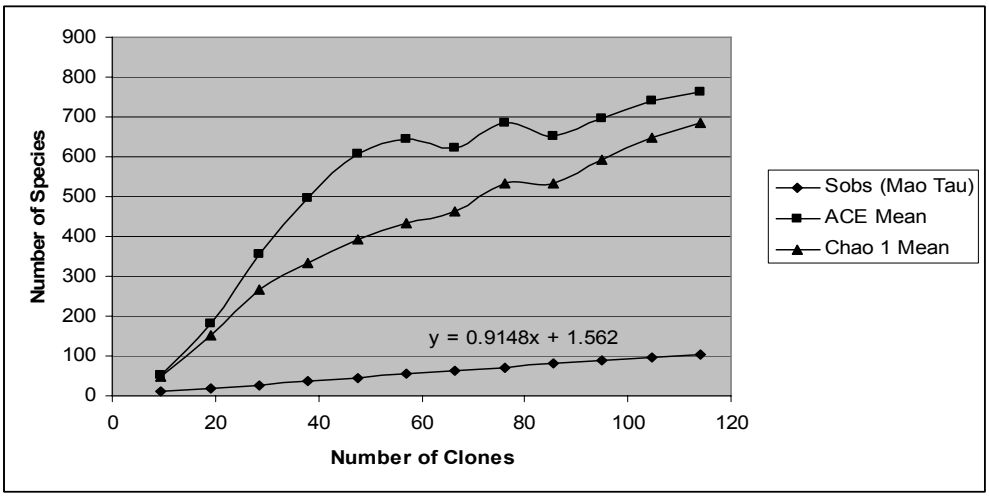


Figure 14. *S. atrovirens* rarefaction curve of number of observed species (S_{obs}) from 16S rRNA gene clone library recovered from mesocosms. Computed by EstimateS. The corrected ACE and Chao1 estimators (after 1,000 randomizations) were also plotted.

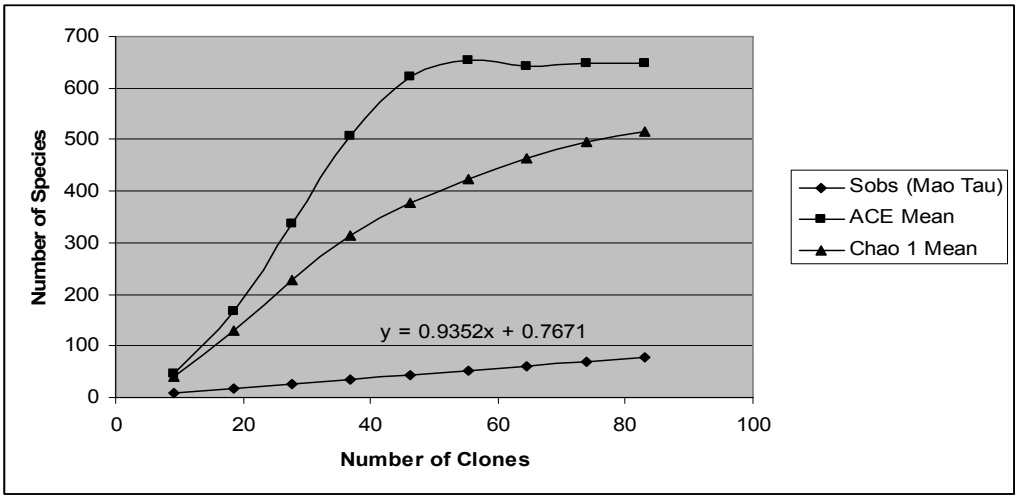


Figure 15. Rarefaction curve of number of observed species (S_{obs}) from 16S rRNA gene clone library recovered from mesocosms with no plantings. Computed by EstimateS. The corrected ACE and Chao1 estimators (after 1,000 randomizations) were also plotted.

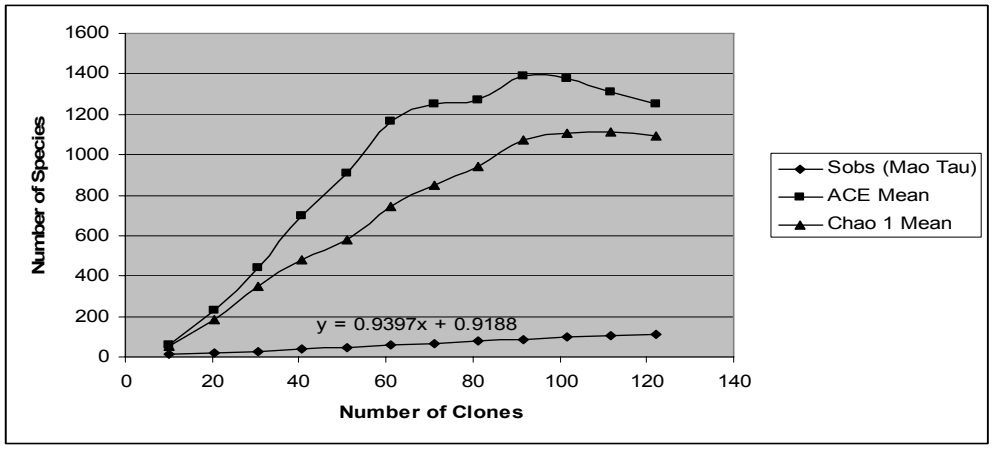


Figure 16. Level 1 (Lowest level) rarefaction curve of number of observed species (S_{obs}) from 16S rRNA gene clone library recovered from mesocosms. Computed by EstimateS. The corrected ACE and Chao1 estimators (after 1,000 randomizations) were also plotted.

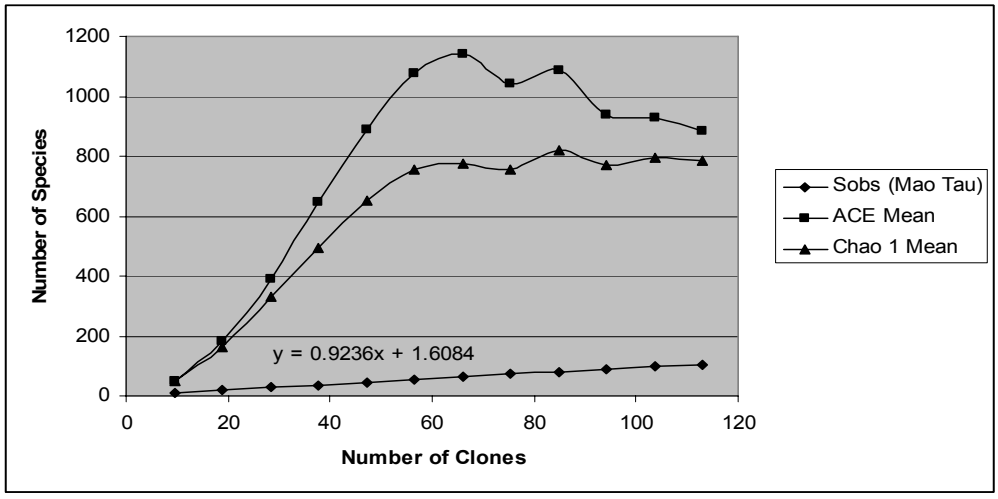


Figure 17. Level 2 (Mid-level) rarefaction curve of number of observed species (S_{obs}) from 16S rRNA gene clone library recovered from mesocosms. Computed by EstimateS. The corrected ACE and Chao1 estimators (after 1,000 randomizations) were also plotted.

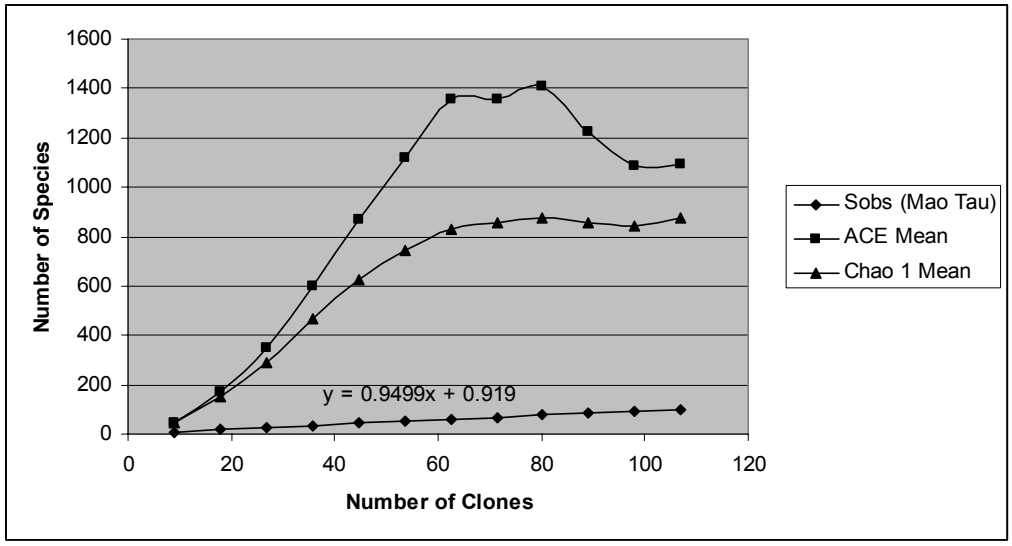


Figure 18. Level 3 (Top level) rarefaction curve of number of observed species (S_{obs}) from 16S rRNA gene clone library recovered from mesocosms. Computed by EstimateS. The corrected ACE and Chao1 estimators (after 1,000 randomizations) were also plotted along with Chao1 95% confidence interval.

As can be seen in Figures 12-18, the number of observed species (S_{obs}) displayed a steady upward slope indicating that sampling was not sufficient. Equations of the related rarefaction curve's trendline was also include to show the average sequences attained per sampling effort. For example, Figure 18 shows a slope of 0.9499 indicating an average of 9.5 unique sequences were attained for every 10 clones. This related closely to the overall Good's coverage of 4.7%,

indicating 9.53 additional phylotypes would be expected for every 10 additional sequenced clones. These levels indicate that the clone library constructed during this research represent only a fraction of the bacterial sequences present in the mesocosms. The ACE and Chao estimators of overall species richness appear to be leveling off or slightly fluctuating and are likely to reach an asymptotic level once rarefaction curve also begins to plateau.

Discussion

The presence of a clone closely matching a known reductive dechlorinator such as *D. ethenogenes* was not found among the sequences. It is likely that the known reductive dechlorinators were numerically low in the samples which resulted in no significant hits. However, several clones did match genera (with a confidence level above 90%) known to have species capable of reduction of chlorinated ethenes. These include *Mycobacterium*, *Geobacter*, and *Nocardioides*. *Dehalobacter* similarity was also shown in sample A52, although at a very low level of confidence (5%). Other genera of note include *Dechlorosoma* (perchlorate reducer) (Achenbach, Michaelidou, Bruce, Fryman, & Coates, 2001) and several sulfate-reducers. RDP Classifier results can be found in Appendix O.

This study serves as a quantitative and qualitative measurement of species diversity. The diversity indices did show an extremely high species diversity, backed up by estimates of richness which have not begun to plateau when applied to a rarefaction curve. This indicates that further sequencing is needed in order to determine phylotype dominance, if any, and to receive insight into the actual abundance of the soil microbial community within the columns as well as to determine if there is a correlation between soil depth/wetland plants and microbial dominance.

V. Conclusion and Recommendations for Further Study

The purpose of this study was to characterize the microbial community and detail microbial dominance, if any, among common wetland hydrophytes at three different strata. This included the construction of mesocosms replicating depths and vegetation parameters of a constructed, up-flow dehalogenating treatment wetland followed by DNA analysis via 16S rDNA PCR and analysis.

The findings indicate that the soil microbial community was much more heterogenous than initially expected and represented bacteria from multiple functional groups. The sampling effort must be increased to the point of reaching enough phylotype duplicates to draw conclusions based both upon depth and planting. It is also obvious from the degree of heterogeneity that no true dominance could be determined with this level of sampling. It is possible, however, to draw some hypothesis from the results such as, "Does S. atrovirens enrich for *Chloroflexi*?"

Effort Strengths

This research provided an initial characterization of the bulk soil microbiota dominated by vegetation from the sedge family. A strong foot-hold is established here, which will provide for useful comparison with follow-on data.

Effort Limitations

This efforts proved weak in providing positive identification of known reductive dehalogenators, possibly due to choice in primers or simply because the abundance of these microbes was very low.

I suggest amplifying 16S rDNA gene using different primers with the same DNA template and pooling different PCR products in order to test the validity of these results and

compare against database results and reduce biases that may be inherent due to primer specificity.

Recommendations for Future Study

Future work evaluating the proper taxonomic placement of the sequenced bacteria would be extremely useful. Closer analysis of the sequences may reveal novel sequences which should be submitted to GenBank.

Additionally, further sampling of the mesocosms within the regions of the rhizoplane and bulk soil post-PCE injection would give more conclusive evidence to the bacterium responsible for dehalogenation. Conducting this study under conditions more representative of field conditions, i.e. simulate temperature, flooding, and photoperiod regimes, would also be helpful. Also construction of columns which include soils rich in organic or iron content may provide results unlike those exhibited here, due to different substrate conditions leading to differing metabolic processes.

The possibility of anomalies such as chimera formations should be examined using programs such as Pintail to verify true diversity amongst the sequences attained. "If one partial-length rDNA fragment of organism A binds to a full- or partial-length rDNA fragment of organism B, one or two full-length chimeric sequences, respectively, can be generated during PCR" (Amann, Ludwig, & Schleifer, 1995). Further amplification of the chimeric sequences proceeds with the same efficiency as for nonchimeric fragments. This may lead to a false interpretation of higher biodiversity in the natural sample. This study would benefit by sorting out chimeric sequences.

This study will be useful in determining PCE degrading microbial community structure once it is corroborated with a post-PCE injection community characterization. Knowledge of the microbial community associated with PCE degradation would provide for proper inoculation of

wetland soils in future remediation efforts. It may serve to answer why remediation efforts at one site differ from another based on microbial communities. It should also prove useful to the design of remediation efforts by giving an indication of whether a site's microbial community will support dechlorination based on the electron donors present.

It is suspected that further study will show that VC dechlorinators will be found in aerobic areas, and root zones may exhibit a high degree of heterogeneity due to the presence of numerous electron donors as well as aerobic and anaerobic conditions capable of supporting multiple organisms.

Appendix A. Acronyms

AFIT:	Air Force Institute of Technology
ATP:	adenosine triphosphate
ATSDR:	Agency for Toxic Substances and Disease Registry
BLAST:	Basic Local Alignment Search Tool
CAH:	Chlorinated aliphatic hydrocarbon
CERCLA:	Comprehensive Environmental Response, Compensation, and Liability Act
CFR:	Code of Federal Regulations
DCE:	dichloroethylene
DNA:	deoxyribonucleic acid
DNAPL:	dense nonaqueous-phase liquid
dsDNA:	double-stranded DNA
EBI:	European Bioinformatics Institute
EDTA:	ethylene diaminetetraacetic acid
EPA:	Environmental Protection Agency
EtBr:	ethidium bromide
LB:	Luria Broth
MCL:	Maximum contaminant level
MMO:	Methane monooxygenase
MNA:	Monitored natural attenuation
NIH:	National Institute of Health
NPL:	National Priority List
NRC:	National Research Council
PCE:	tetrachloroethylene
PCR:	Polymerase Chain Reaction
RCRA:	Resource Conservation and Recovery Act
rDNA:	ribosomal DNA
RDP:	Ribosomal Database Project
TAE:	Tris-acetate EDTA
TCA:	trichloroethane
TCE:	trichloroethylene
USDHHS:	U.S. Department of Health and Human Services
UV:	ultraviolet
VC:	vinyl chloride
VOC:	volatile organic compound
WPAFB:	Wright-Patterson Air Force Base
WSU:	Wright State University

Appendix B. Previously Identified Dechlorinating Microbes

Identified Dechlorinating Microbes					
Chlorinated Compound/Electron Acceptors	**Aerobe**	**Anaerobe**	**End-Product of Dechlorination**	**Metabolic Pathway**	**Optimum Temp.**
PCE		*Dehalobacter restrictus* [g]	*cis* -1,2-DCE	dehalorespiration	25-30 °C
		Desulfitobacterium chlororespirans [j]	<5% of PCE consumed; small amounts cis- and trans-1,2-DCE	dehalorespiration	34-38 °C
		Desulfitobacterium dehalogenans [k]	*cis* -1,2-DCE	dehalorespiration	nr
		Dehalospirillum multivorans [l]	*cis* -1,2-DCE	dehalorespiration	30 °C
		Dehalococcoides ethenogenes [f]	ethene	dehalorespiration	35 °C
		Desulfomonile tiedjei [h]	*cis* -1,2-DCE	dehalorespiration	nr
TCE		*Dehalobacter restrictus* [g]	*cis* -1,2-DCE	dehalorespiration	25-30 °C
		Dehalococcoides ethenogenes [f]	ethene	dehalorespiration	35 °C
		Desulfitobacterium hafniense [i]			
		Desulfitobacterium chlororespirans [j]	cis- and trans-1,2-DCE	dehalorespiration	34-38 °C
	Methanotrophs (*Methylomonas* sp., *Methylosinus* sp.) [e]		CO_2	Cometabolic Oxidation	varies
	Rhodococcus sp. [d]		CO_2	Oxidation	4-35 °C
	Nitrosomonas europaea [a]		CO_2	Cometabolic Oxidation	nr
	Pseudomona sp. [e]		CO_2	Cometabolic Oxidation	nr
	Methylocystis parvus [h]		CO_2	Cometabolic Oxidation	nr
	Mycobacterium sp [h]		nr	Cometabolic Oxidation	nr
	Alcaligenes eutrophus [h]		nr	Cometabolic Oxidation	nr
1,1-DCE	*Mycobacterium aurum* [c]		chlorooxirane	Cometabolic Oxidation	nr
	Rhodococcus sp. [d]		CO_2	Cometabolic Oxidation	4-35 °C
	Pseudomona sp. [e]		CO_2		nr
		Dehalococcoides ethenogenes [f]	ethene	dehalorespiration	35 °C
		Geobacter sp. [a]	Iron-reducing role in oxidation	Oxidation	nr
cis-1,2 -DCE	*Mycobacterium aurum* [c]		chlorooxirane	Oxidation	nr
	Rhodococcus sp. [D]		CO_2	Oxidation	nr
	Nitrosomonas europaea [a]		CO_2	Cometabolic Oxidation	nr
	Pseudomona sp. [e]		CO_2		nr
		Dehalococcoides ethenogenes [f]	ethene	dehalorespiration	35 °C
		Geobacter sp. [a]	Iron-reducing role in oxidation	Oxidation	nr
	Methanotrophs (*Methylomonas* sp., *Methylosinus* sp.) [e]		CO_2	Cometabolic Oxidation	varies
trans-1,2-DCE	*Mycobacterium aurum* [c]		chlorooxirane	Cometabolic Oxidation	nr
	Nitrosomonas europaea [a]		CO_2	Cometabolic Oxidation	nr
	Pseudomona sp. [e]		CO_2		nr
	Methanotrophs (*Methylomonas* sp., *Methylosinus* sp.) [e]		CO_2	Cometabolic Oxidation	varies
		Dehalococcoides ethenogenes [f]	ethene	dehalorespiration	35 °C
		Geobacter sp. [a]	Iron-reducing role in oxidation	Oxidation	
VC	*Mycobacterium aurum* [c]		chlorooxirane	Cometabolic Oxidation	nr
	Rhodococcus sp. [d]		CO_2	Oxidation	4-35 °C
	Actinomycetales sp. [a]		CO_2	Oxidation	nr
	Nitrosomonas europaea [a]		CO_2	Cometabolic Oxidation	nr
		Dehalococcoides ethenogenes [f]	ethene	dehalorespiration	35 °C
		Geobacter sp. [a]	Iron-reducing role in oxidation	Oxidation	nr
	Methanotrophs (*Methylomonas* sp., *Methylosinus* sp.) [e]		CO_2	Cometabolic Oxidation	varies
	Nocardioides sp. [b]		CO_2	Oxidation	nr

Source: a=cited in Lee et al.(1998); b= Coleman (2002); c= Hartmans and De Bont (1992); d=Phelps et al. (1991); e=Ensley (1991); f=Maymo-Gatell et al. (1999); g=Holliger (1992); h=Wackett (1995); i=Neumann (1994); Sanford et al. (1996); k=Utkin et al. (1994); l=Christiansen and Ahring (1996); nr=not reported

Appendix C. Mo Bio PowerSoil™ DNA Isolation Kit Extraction Protocol. (Mo Bio Laboratories, Carlsbad, CA, 2004)

Introduction

The PowerSoil™ DNA Isolation Kit is comprised of a novel and proprietary method for isolating genomic DNA from environmental samples. The kit is intended for use with environmental samples containing a high humic acid content including difficult soil types such as compost, sediment, and manure. Other more common soil types have also been used successfully with this kit. The isolated DNA has a high level of purity allowing for more successful PCR amplification of organisms from the sample. PCR analysis has been performed to detect a variety of organisms including bacteria (e.g. *Bacillus subtilis, Bacillus anthracis*), fungi (e.g. yeasts, molds), algae and Actinomycetes (e.g. *Streptomyces*).

The PowerSoil DNA Kit distinguishes itself from Mo Bio's UltraClean™ Soil DNA Isolation Kit with a **NEW** humic substance/brown color removal procedure. This new procedure is effective at removing PCR inhibitors from even the most difficult soil types.

Environmental samples are added to a bead beating tube for rapid and thorough homogenization. Cell lysis occurs by mechanical and chemical methods. Total genomic DNA is captured on a silica membrane in a spin column format. DNA is then washed and eluted from the membrane. DNA is then ready for PCR analysis and other downstream applications.

WARNING: Solution C5 contains ethanol. It is flammable.

IMPORTANT NOTE FOR USE: Make sure the 2 ml PowerBead Tubes rotate freely in your centrifuge without rubbing.

Kit Storage

Kit reagents and components should be stored at room temperature.

Kit Contents

	Quantity	
Component	**12888-50**	**12888-100**
PowerBead Tubes (contain 750 ul solution)	50	100
Solution C1	3.3 ml	6.6 ml
Solution C2	14 ml	28 ml
Solution C3	11 ml	22 ml
Solution C4	72 ml	144 ml
Solution C5	27.5 ml	55 ml
Solution C6	6 ml	12 ml
Spin Filter Units in 2 ml Tubes	50	100
Collection Tubes (2 ml)	200	400

1. To the 2ml PowerBead Tubes provided, add 0.25 gm of soil sample.
2. Gently vortex to mix.
3. **Check Solution C1.** If Solution C1 is precipitated, heat solution to 60°C until dissolved before use.
4. Add 60µl of Solution C1 and invert several times or vortex briefly.
5. Secure PowerBead Tubes horizontally using the Mo Bio Vortex Adapter tube holder for the vortex (Mo Bio Catalog No. 13000-V1. Call 1-800-606-6246 for information) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes.
6. Make sure the PowerBead Tubes rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x *g* for 30 seconds. **CAUTION:** Be sure not to exceed 10,000 x g or tubes may break.
7. Transfer the supernatant to a clean microcentrifuge tube (provided).
   Note: Expect between 400 to 500µl of supernatant. Supernatant may still contain some soil particles.
8. Add 250µl of Solution C2 and vortex for 5 seconds. Incubate at 4°C for 5 minutes.
9. Centrifuge the tubes for 1 minute at 10,000 x *g.*
10. Avoiding the pellet, transfer up to, but no more than, 600µl of supernatant to a clean microcentrifuge tube (provided).
11. Add 200µl of Solution C3 and vortex briefly. Incubate at 4°C for 5 minutes.
12. Centrifuge the tubes for 1 minute at 10,000 x *g.*
13. Avoiding the pellet, transfer up to, but no more than, 750µl of supernatant into a clean microcentrifuge tube (provided).
14. Add 1200µl of Solution C4 to the supernatant and vortex for 5 seconds.
15. Load approximately 675µl onto a spin filter and centrifuge at 10,000 x *g* for 1 minute. Discard the flow through and add an additional 675µl of supernatant to the spin filter and centrifuge at 10,000 x *g* for 1 minute. Load the remaining supernatant onto the spin filter and centrifuge at 10,000 x *g* for 1 minute. **Note**: A total of three loads for each sample processed are required.
16. Add 500µl of Solution C5 and centrifuge for 30 seconds at 10,000 x *g*.
17. Discard the flow through.
18. Centrifuge again for 1 minute.
19. Carefully place spin filter in a new clean tube (provided). Avoid splashing any Solution C5 onto the spin filter.
20. Add 100µl of Solution C6 to the center of the white filter membrane. Alternatively, sterile DNA-Free PCR Grade Water may be used for elution from the silica spin filter membrane at this step (Mo Bio Catalog No. 17000-10).
21. Centrifuge for 30 seconds.
22. Discard the spin filter. DNA in the tube is now application ready. No further steps are required.
    We recommend storing DNA frozen (-20° to -80°C). Solution C6 contains no EDTA.

Wet Soil Sample

If soil sample is high in water content, remove contents from PowerBead Tube (beads and solution) and transfer into another sterile microcentrifuge tube (not provided). Add soil sample to PowerBead Tube and centrifuge for 30 seconds at 10,000 x g. Remove as much liquid as

possible with a pipet tip. Add beads and bead solution back to PowerBead Tube and follow protocol starting at step 2.

If DNA Does Not Amplify

- Make sure to check DNA yields by gel electrophoresis or spectrophotometer reading. An excess amount of DNA will inhibit a PCR reaction.
- Diluting the template DNA should not be necessary with DNA isolated with the PowerSoil DNA Isolation Kit; however, it should still be attempted.
- If DNA will still not amplify after trying the steps above, then PCR optimization (changing reaction conditions and primer choice) may be needed.

Eluted DNA Sample Is Brown

We have not observed any coloration in DNAs isolated using the PowerSoil DNA Isolation kit. If you observe coloration in your samples, please contact technical support for suggestions.

Alternative Lysis Method

After adding Solution C1, vortex 3-4 seconds, then heat to 70°C for 5 minutes. Vortex 3-4 seconds. Heat another 5 minutes. Vortex 3-4 seconds. This alternative procedure will reduce shearing but may also reduce yield.

Concentrating the DNA

Your final volume will be 100µl. If this is too dilute for your purposes, add 4µl of 5M NaCl and mix. Add 200µl of 100% cold ethanol and mix. Centrifuge at 10,000 x g for 5 minutes. Decant all liquid. Dry residual ethanol in a speed vac, dessicator, or air dry. Resuspend precipitated DNA in desired volume.

DNA Floats Out of Well When Loaded on a Gel

You may have inadvertently transferred some residual Solution C5 into the final sample. Prevent this by being careful in step 19 not to transfer liquid onto the bottom of the spin filter basket. Ethanol precipitation is the best way to remove Solution C5 residue. (See "Concentrating the DNA" above)

Storing DNA

DNA is eluted in Solution C6 (10mM Tris) and must be stored at -20° to 80°C or it may degrade over time. DNA can be eluted in TE but the EDTA may inhibit reactions such as PCR and automated sequencing. DNA may also be eluted with sterile DNA-Free PCR Grade Water (Mo Bio Catalog No. 17000-10).

Cells are Difficult to Lyse

If cells are difficult to lyse, a 10 minute incubation at 70°C, after adding Solution C1, can be performed. Follow by continuing with protocol step 5.

Technical Information

Product Manufactured by Mo Bio Laboratories, Inc. 2746 Loker Avenue West, Carlsbad, CA 92008

Appendix D. Polymerase Chain Reaction & PCR Protocol Using HotStarTaq Master Mix.

Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a repetitive amplifying technique used to copy targeted DNA 10^6 times or greater. This allows for detection of unculturable microbes and for pinpointing specific genes of concern. PCR is a multi-step process requiring strict adherence to documented protocol. PCR relies on polymerase enzymes to copy a target DNA sequence repeatedly during a series of 25-35 heating/cooling cycles. Each cycle denatures, anneals, and extends targeted DNA segments, resulting in an exponential increase in DNA. In theory, 30 cycles would result in a 2^{30} increase, however, due to intrinsic inefficiencies a 10^6 –fold increase is typical (Maier, Pepper, & Gerba, 2000). The PCR product is visualized using gel electrophoresis (see Figure 20).

When two complimentary strands of DNA come together to form a double strand (dsDNA) the process is known as hybridization. The opposite reaction, in which dsDNA is split by heating to 94° C for 1 minute, is called denaturation. Upon cooling, these split strands will hybridize back again, a process known as reannealing (Maier, Pepper, & Gerba, 2000).

The first step involves mixing and adding the "master mix" to extracted DNA in a microcentrifuge tube. Master mix contains a PCR mixture of buffers, nucleotides (adenine (A), guanine (G), thymine (T), and cytosine(C)), and thermostable polymerase. Primers are also added during this step to allow for the targeting of a specified region of small subunit rDNA. Initial heating to 95° C is then done in order to activate the *Taq* polymerase. DNA is subsequently denatured at 94° C. Once denaturation takes place, temperatures are lowered to a range typically between 50-70° C allowing for primer attachment.

Primers are oligonucleotides - short segments of single-stranded DNA with a complementary sequence to a targeted region of DNA. The primers anneal to the denatured

DNA and allow amplification of DNA to occur in the region defined by the "upstream" and "downstream" primers (Qiagen, 2002).

The final step of PCR is extension. During the extension phase, DNA polymerase copies the targeted strand of DNA by adding complementary bases from the 3' end of primers. PCR results in a dsDNA molecule identical to the original. During extension *Taq* polymerase is typically used due to its intrinsic heat resistance. *Taq* polymerase is an enzyme obtained from the thermophilic bacterium *Thermus aquaticus,* isolated from a hot spring in Yellowstone National Park. It is also stable, therefore able to be used through many cycles. For extensions under 1000bp (base pairs), the extension step is complete in approximately a minute at 72° C (Qiagen, 2002), the ideal temperature for the polymerase enzyme. At high temperatures, nonspecific hybridization is rare, thus making the product of *Taq* PCR more homogeneous than that obtained using the *E. coli* enzyme. This process is repeated 25 to 35 times using a thermocycler which automates the heating/cooling cycle. The entire cycling process typically takes 3-5 hours to complete. The amplified product is then analyzed using agarose gel

electrophoresis prior to cloning in order to ensure adequate DNA amplification has occurred.

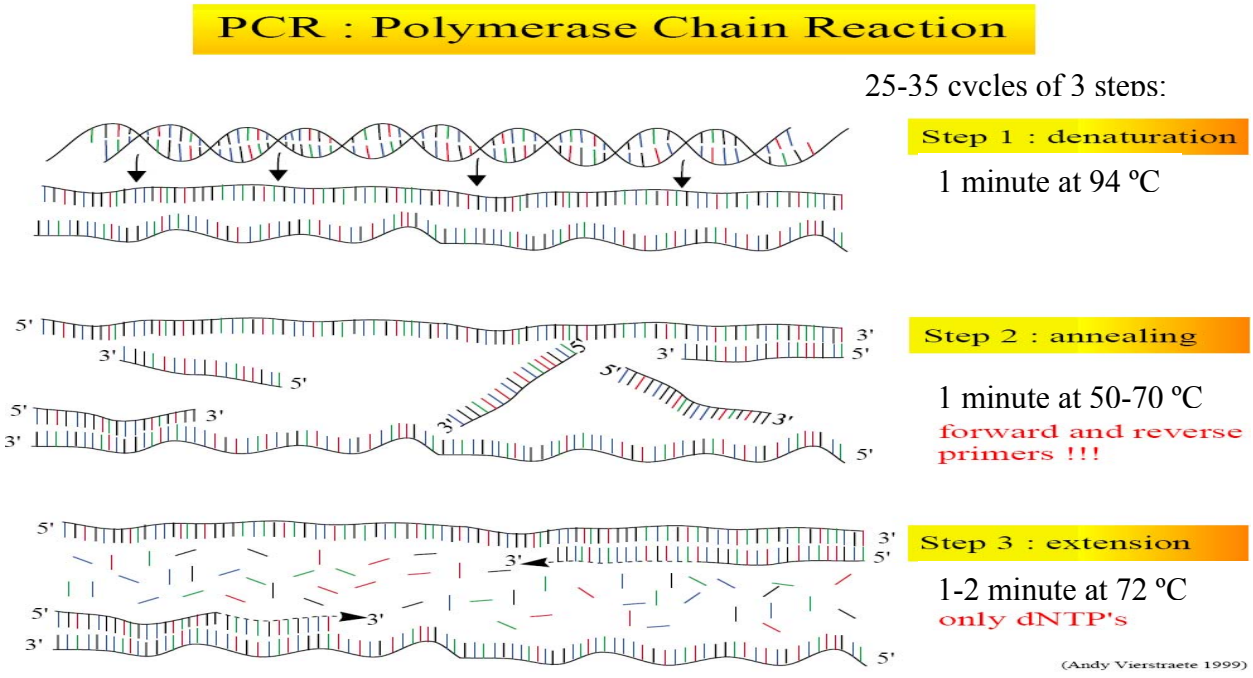


Figure 19. Principle Steps of PCR. (Adapted from Vierstraete, 1999)

PCR Protocol Using HotStarTaq Master Mix. (Qiagen, 2002)

This protocol serves only as a guideline for PCR amplification. Optimal reaction conditions, such as incubation times and temperatures, and amount of template DNA, may vary and need to be determined individually.

Notes:

- **Each PCR program should be started with an initial activation step of 15 min at 95°C to activate HotStarTaq DNA Polymerase (see step 6 of this protocol).**
- HotStarTaq Master Mix provides a final concentration of 1.5 mM $MgCl_2$ in the final reaction mix, which will produce satisfactory results in most cases. However, if a higher Mg^{2+} concentration is required, prepare a stock solution containing 25 mM $MgCl_2$.
- Set up reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.

1. Thaw primer solutions.

Mix well before use.

Optional: prepare a primer mix of an appropriate concentration (see Table 4) using the water provided.
This is recommended if several amplification reactions using the same primer pair are to be performed. The final volume of diluted primer mix should be 25 µl per reaction including the template DNA, added at step 4.

2. Mix the HotStarTaq Master Mix by vortexing briefly and dispense 25 µl into each PCR tube according to Table 4.

It is important to mix the HotStarTaq Master Mix before use in order to avoid localized concentrations of salt. HotStarTaq Master Mix is provided as a 2x concentrate (i.e., a 25 µl volume of the HotStarTaq Master Mix is required for amplification reactions with a final volume of 50 µl). For volumes smaller than 50 µl, the 1:1 ratio

of HotStarTaq Master Mix to diluted primer mix and template should be maintained as defined in Table 4. A negative control (without template DNA) should always be included. It is not necessary to keep PCR tubes on ice as nonspecific DNA synthesis cannot occur at room temperature due to the inactive state of HotStarTaq DNA Polymerase.

3. Distribute the appropriate volume of diluted primer mix into the PCR tubes containing the Master Mix.

4. Add template DNA (ϒ⇐1µg/reaction) to the individual PCR tubes.
The volume added should not exceed 10% of the final PCR volume.

Table 4. Reaction composition using HotStarTaq Master Mix

Component	Volume/reaction	Final concentration
HotStarTaq Master Mix 25 µl	25 µl	2.5 units HotStarTaq DNA Polymerase 1x PCR Buffer* 200 µM of each dNTP
Diluted primer mix		
Primer A 0.1–0.5 µM	Variable	0.1–0.5 µM
Primer B	Variable	0.1–0.5 µM
Distilled water (provided)	Variable	–
Template DNA		
Template DNA, added at step 4	Variable	≤1 µg/reaction
Total volume	50 µl	–

**Contains 1.5 mM $MgCl_2$*

5. When using thermal cyclers with a heated lid, do not use mineral oil. Proceed directly to step 6. Otherwise, overlay with approximately 50 µl mineral oil.

6. Program the thermal cycler according to the manufacturer's instructions.
Each PCR program must start with an initial heat activation step at 95°C for 15 min. A typical PCR cycling program is outlined below. For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target and primer pair.

			Additional comments
Initial activation step:	15 min	95°C	HotStarTaq DNA Polymerase is activated by this heating step.
3-step cycling			
Denaturation:	0.5–1 min	94°C	
Annealing:	0.5–1 min	50–68°C	5°C below Tm of primers
Extension:	1 min	72°C	For PCR products longer than 1 kb, use an extension time of approximately 1 min per kb DNA.
Number of cycles:	25–35		
Final extension:	10 min	72°C	

7. Place the PCR tubes in the thermal cycler and start the cycling program.
Note: After amplification, samples can be stored overnight at 2–8°C or at –20°C for longer storage.

Appendix E. Preparing and Running Gel

1. **Mix TAE Buffer**
    a. Make a Tris-acetate EDTA (TAE) solution. This solution comes from WSU at a 50X concentration and is prepared with following recipe:

        Add the following to 900ml distilled H_2O:

        - 242g Tris base.
        - 57.1ml Glacial Acetic Acid.
        - 18.6 g EDTA

        Adjust volume to 1L with additional distilled H_2O.

    b. Dilute to a 1X concentration. The formula for this is

$$C_1 * V_1 = C_2 * V_2$$

    c. For this requirement, we would like to get 1000 ml of TAE at a concentration of 1X so we plug in the known values to get the volume of TAE @ 50X concentration and then subtract that from the overall 1000 mL to get the amount of distilled water.

    d. Plug in known values and solve for V_2:

$$\frac{(1X)*(1000ml)}{(50X)} = 20ml = \mathrm{V}_2$$

    e. Subtract the V_2 from the overall solution that we want to make and that gives the amount of distilled water that we need which to make a 1000ml 1X TAE solution of. This means we need 980ml of DI H_20 and 20ml of TAE 1X.

2. **Mixing Agarose Gel** (for small gel box; adjust by a factor of 1.5 for large tray).
    a. Make a 70ml of an 0.8% Agarose solution (1% used for plasmid DNA imaging).
    b. Mix 0.56g (0.7g for 1%) of Agarose into 70ml of TAE 1X solution
    c. Mix and place into microwave
    d. Microwave on high for about 1.5 to 2.0 minutes to bring solution to boil.
    e. Once Agarose is dissolved remove from microwave and allow to cool to touch.
    f. Add $70\mu l$ of EtBr 100X and swirl
        i. **General Information:** Ethidium Bromide (EtBr) is a commonly used stain for the visualization of nucleic acids in agarose gels. It is widely used by scientists due to its high sensitivity, rapid staining and very inexpensive price. While it is not specifically regulated as a hazardous waste EtBr is a suspected carcinogen. The mutagenic properties may present a hazard if it is not managed properly in the laboratory.

ii. **Personal Protection:** When handling EtBr always wear a lab coat, nitrile gloves, and chemical splash goggles. Proper skin and eye protection are needed when a ultraviolet (UV) light source is used while working with EtBr. Avoid exposing unprotected skin and eyes to intense UV sources. Wear a face shield if UV source is pointing upwards. When working with a UV source for a long time, wrap up lab coat sleeves with tape or other means where the wrist could be exposed.

iii. **Disposal of EtBr**: Electrophoresis Gels: Trace amounts of EtBr (less than 0.1%) in electrophoresis gels do not pose a serious hazard so they can be discarded in the trash if properly bagged and secured. If the gels contain more than 0.1% EtBr they should be placed in an appropriate container for hazardous waste disposal. Environmental Health and Safety (EH&S) has a variety of containers that are available to collect and dispose of gels.

g. Place comb in gel box
h. Add the 70ml of solution to the Gel Bed and allow hardening approximately 30 minutes.
i. Once gel is hardened, remove comb from gel bed.
j. Extract gel bed from gel box and rotate 90 degrees so that wells formed by the comb are opposite the red (Pos) leads.
k. Fill Gel box with 1X TAE until both sides of gel box overflow and the level of buffer is flush with top of hardened gel.

3. Prepare PCR Samples for Gel

a. Take PCR sample and remove 3 into a autoclaved Eppendorf tube
b. 17 to 18.5 μl of distilled water depending on buffer concentrations of 6X and 10X, respectively.
c. 2.4μl or 4μl of 10X or 6X buffer, respectively (does not have to be refrigerated).

4. Load DNA into Wells and Run GEL

a. Add 6μl of 1kb DNA Ladder (or λ ladder for visualizing plasmid DNA) into the 1st well (Toward the Black (-) Lead)
b. Add 24μl of PCR samples for Gel prepared in step 3
c. Attach colored leads to matching receptors on Gel box and power source.
d. Turn on power source and allow to run at 95V until there is a clear separation (Approximately 35 minutes).

5. Imaging of GEL via Kodak Gel Logic 200

Appendix F. PCR Data & Results

	Extract #	Sample Extraction Date	DNA Extraction Date	PCR #1									Gel #1				
				Primers				Master Mix* (µl)	H20 (µl)	Template DNA (µl)	Annealing Temp (°C)	PCR Product (µl)	H20 (µl)	Gel Buffer		PCR #1 (date)	PCR #1 Results
				Forward Primer	Vol. (µl)	Reverse Primer	Vol. (µl)							Conc.	Vol. (µl)		
Soil+Inoculum	SI5	17-May-05	30-Jun-05	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	23-Sep-05	+
	SI6	17-May-05	30-Jun-05	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	23-Sep-05	+
Column 1 (C. comosa)	A11	23-Aug-05	9-Sep-05	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	17	6X	4	12-Sep-05	+
	A12	23-Aug-05	9-Sep-05	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	17	6X	4	12-Sep-05	+
	A13	23-Aug-05	9-Sep-05	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	17	6X	4	12-Sep-05	+/-
Column 2 (C. comosa)	A21	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
	A22	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
	A23	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
Column 3 (Blank)	A31	23-Aug-05	9-Sep-05	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	17	6X	4	12-Sep-05	-
	A32	23-Aug-05	9-Sep-05	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	17	6X	4	12-Sep-05	-
	A33	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	20-Sep-05	-
Column 4 (E. erythropoda)	A41	23-Aug-05	9-Sep-05	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	17	6X	4	12-Sep-05	-
	A42	23-Aug-05	9-Sep-05	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	17	6X	4	12-Sep-05	+
	A43	23-Aug-05	9-Sep-05	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	17	6X	4	12-Sep-05	+
Column 5 (S. atrowirens)	A51	23-Aug-05	9-Sep-05	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	17	6X	4	12-Sep-05	-
	A52	23-Aug-05	9-Sep-05	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	17	6X	4	12-Sep-05	+
	A53	23-Aug-05	9-Sep-05	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	17	6X	4	12-Sep-05	+
Column 6 (S. atrowirens)	A61	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
	A62	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
	A63	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
Column 7 (E. erythropoda)	A71	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
	A72	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
	A73	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
Column 8 (Blank)	A81	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
	A82	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
	A83	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
Column 9 (S. atrowirens)	A91	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
	A92	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
	A93	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
Column 10 (E. erythropoda)	A10-1	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
	A10-2	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
	A10-3	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
Column 11 (Blank)	A11-1	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
	A11-2	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
	A11-3	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
Column 12 (S. atrowirens)	A12-1	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
	A12-2	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
	A12-3	23-Aug-05	14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
Blank			30-Jun-05														
Blank			9-Sep-05	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	17	6X	4	12-Sep-05	-
Blank			14-Sep-05	E8F	1.0	E533R	1.0	12.5 (Gm)	0	10.5	46	3	18.5	10X	2.4	19-Sep-05	-
Blank			14-Sep-05									3	18.5	10X	2.4	23-Sep-05	+

Note: For extraction nomenclature Letter is month of extraction; 1st number represents column; 2nd number represents depth, with 1,2, and 3 representing top, middle, and bottom, respectively. (e.g. A=August, 1=Column 1; 2=Middle Depth)

*Mastermix is Hotstart unless anotated by"Gm"

		PCR #2								Gel #2						
		Primers				Master						Gel Buffer				
	Extract #	Forward Primer	Vol. (µl)	Reverse Primer	Vol. (µl)	Mix* (µl)	H20 (µl)	Template DNA (µl)	Annealing Temp (°C)	PCR Product (µl)	H20 (µl)	Conc.	Vol. (µl)	PCR #2 (date)	PCR #2 Results	Comments
Soil+Inoculum	SI5	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	-	
	SI6	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	-	
Column 1 (C. comosa)	A11	E8F	1.0	E533R	1.0	12.5 (Gm)	5	5.5	46	3	18.5	10X	2.4	21-Sep-05	-	GO Taq
	A12	E8F	1.0	E533R	1.0	12.5 (Gm)	5	5.5	46	3	18.5	10X	2.4	21-Sep-05	-	GO Taq
	A13	E8F	1.0	E533R	1.0	12.5 (Gm)	5	5.5	46	3	18.5	10X	2.4	21-Sep-05	-	GO Taq
Column 2 (C. comosa)	A21	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	20-Sep-05	+/-	HotstarTaq used
	A22	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	20-Sep-05	+	HotstarTaq used
	A23	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	20-Sep-05	+/-	HotstarTaq used
Column 3 (Blank)	A31	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	17	6X	4	13-Sep-05	-	
	A32	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	17	6X	4	13-Sep-05	-	
	A33	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	+	
Column 4 (E. erythropoda)	A41	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	17	6X	4	13-Sep-05	-	
	A42	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	17	6X	4	13-Sep-05	+	
	A43	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	17	6X	4	13-Sep-05	+	
Column 5 (S. atrovirens)	A51	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	17	6X	4	13-Sep-05	-	
	A52	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	17	6X	4	13-Sep-05	+	
	A53	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	17	6X	4	13-Sep-05	+	
Column 6 (S. atrovirens)	A61	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	+/-	
	A62	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	+	
	A63	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	+	
Column 7 (E. erythropoda)	A71	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	+	
	A72	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	+/-	
	A73	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	+/-	
Column 8 (Blank)	A81	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	+	
	A82	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	+	
	A83	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	-	
Column 9 (S. atrovirens)	A91	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	+	
	A92	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	+	
	A93	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	-	
Column 10 (E. erythropoda)	A10-1	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	-	
	A10-2	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	-	
	A10-3	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	-	
Column 11 (Blank)	A11-1	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	-	
	A11-2	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	+/-	
	A11-3	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	+/-	
Column 12 (S. atrovirens)	A12-1	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	23-Sep-05	+	
	A12-2	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	23-Sep-05	+/-	
	A12-3	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	23-Sep-05	+	
Blank		E8F	1.0	E533R												
Blank		E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	17	6X	4	12-Sep-05	-	
Blank																
Blank																

		PCR #3								Gel #3						
		Primers				Master				PCR		Gel Buffer				
	Extract #	Forward Primer	Vol. (µl)	Reverse Primer	Vol. (µl)	Mix * (µl)	H20 (µl)	Template DNA (µl)	Annealing Temp (°C)	Product (µl)	H20 (µl)	Conc.	Vol. (µl)	PCR #3 (date)	PCR #3 Results	Comments
Soil+Inoculum	SI5	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	3-Oct-05	+	Complete
	SI6	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	3-Oct-05	+	Complete
Column 1 (C. comosa)	A11	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	23-Sep-05	+	Complete
	A12	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	23-Sep-05	+	Complete
	A13	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	23-Sep-05	+	
Column 2 (C. comosa)	A21	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	23-Sep-05	+	
	A22	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	23-Sep-05	+	
	A23	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	23-Sep-05	+	
Column 3 (Blank)	A31	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	-	
	A32	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	-	
	A33	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	3-Oct-05	+	Complete
Column 4 (E. erythropoda)	A41	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	29-Sep-05	+	
	A42	E8F	1.0	E533R	1.0	12.5	0.0	10.5	46	3	18.5	10X	2.4	9-Nov-05	-	
	A43	E8F	1.0	E533R	1.0	12.5	3.0	7.5	46	3	18.5	10X	2.4	9-Nov-05	+	Complete
Column 5 (S. atrovirens)	A51	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	29-Sep-05	+	
	A52	E8F	1.0	E533R	1.0	12.5	3.0	7.5	46	3	18.5	10X	2.4	9-Nov-05	+/-	Complete
	A53	E8F	1.0	E533R	1.0	12.5	3.0	7.5	46	3	18.5	10X	2.4	9-Nov-05	+	Lost 12Sept sa
Column 6 (S. atrovirens)	A61	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	3-Oct-05	-	
	A62	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	3-Oct-05	+	Complete
	A63	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	3-Oct-05	+	Complete
Column 7 (E. erythropoda)	A71	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	3-Oct-05	+	Complete
	A72	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	3-Oct-05	+	Complete
	A73	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	3-Oct-05	+	Complete
Column 8 (Blank)	A81	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	3-Oct-05	+	Complete
	A82	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	3-Oct-05	+	Complete
	A83	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	3-Oct-05	+	
Column 9 (S. atrovirens)	A91	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	3-Oct-05	+	Complete
	A92	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	3-Oct-05	+	Complete
	A93	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	3-Oct-05	+	
Column 10 (E. erythropoda)	A10-1	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	3-Oct-05	+	
	A10-2	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	3-Oct-05	+	
	A10-3	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	3-Oct-05	+	
Column 11 (Blank)	A11-1	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	3-Oct-05	+	
	A11-2	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	3-Oct-05	+	Complete
	A11-3	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	3-Oct-05	+	Complete
Column 12 (S. atrovirens)	A12-1	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	+	Complete
	A12-2	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	29-Sep-05	+	
	A12-3	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	+	Complete

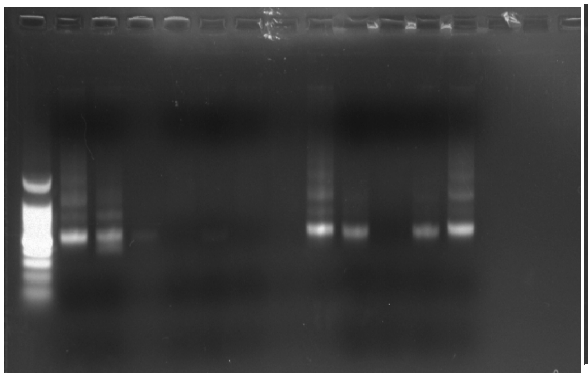
		PCR #4								Gel #4						
		Primers				Master						Gel Buffer				
	Extract #	Forward Primer	Vol. (µl)	Reverse Primer	Vol. (µl)	Mix * (µl)	H20 (µl)	Template DNA (µl)	Annealing Temp (°C)	PCR Product (µl)	H20 (µl)	Conc.	Vol. (µl)	PCR #4 (date)	PCR #4 Results	Comments
Soil+Inoculum	SI5															
	SI6															
Column 1 (C. comosa)	A11															
	A12															
	A13	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	+/-	
Column 2 (C. comosa)	A21	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	-	
	A22	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	+	Complete
	A23	E8F	1.0	E533R	1.0	12.5	0	10.5	46	3	18.5	10X	2.4	29-Sep-05	+	Complete
Column 3 (Blank)	A31	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	3-Oct-05	+	
	A32	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	3-Oct-05	-	
	A33															
Column 4 (E. erythropoda)	A41	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	3-Oct-05	+	Complete
	A42	E8F	1.0	E533R	1.0	12.5	5.0	5.5	46	3	18.5	10X	2.4	16-Nov-05	+	Complete
	A43															
Column 5 (S. atrovirens)	A51	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	3-Oct-05	-	
	A52															
	A53	E8F	1.0	E533R	1.0	12.5	5.0	5.5	46	3	18.5	10X	2.4	17-Nov-05	+	Complete
Column 6 (S. atrovirens)	A61	E8F	1.0	E533R	1.0	12.5	0.0	10.5	46	3	18.5	10X	2.4	9-Nov-05	+/-	
	A62															
	A63															
Column 7 (E. erythropoda)	A71															
	A72															
	A73															
Column 8 (Blank)	A81															
	A82															
	A83	E8F	1.0	E533R	1.0	12.5	5.0	5.5	46	3	18.5	10X	2.4	9-Nov-05	+	Complete
Column 9 (S. atrovirens)	A91															
	A92															
	A93	E8F	1.0	E533R	1.0	12.5	5.0	5.5	46	3	18.5	10X	2.4	9-Nov-05	+	Complete
Column 10 (E. erythropoda)	A10-1	E8F	1.0	E533R	1.0	12.5	5.0	5.5	46	3	18.5	10X	2.4	9-Nov-05	+	Complete
	A10-2	E8F	1.0	E533R	1.0	12.5	5.0	5.5	46	3	18.5	10X	2.4	9-Nov-05	+	Complete
	A10-3	E8F	1.0	E533R	1.0	12.5	5.0	5.5	46	3	18.5	10X	2.4	9-Nov-05	+	Complete
Column 11 (Blank)	A11-1	E8F	1.0	E533R	1.0	12.5	5.0	5.5	46	3	18.5	10X	2.4	9-Nov-05	+	Complete
	A11-2															
	A11-3															
Column 12 (S. atrovirens)	A12-1															
	A12-2	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	3-Oct-05	+	Complete
	A12-3															

	Extract #	PCR #5								Gel #5						Comments
		Primers				Master Mix * (µl)	H20 (µl)	Template DNA (µl)	Annealing Temp (°C)	PCR Product (µl)	H20 (µl)	Gel Buffer		PCR #5 (date)	PCR #5 Results	
		Forward Primer	Vol. (µl)	Reverse Primer	Vol. (µl)							Conc.	Vol. (µl)			
Soil+Inoculum	SI5															
	SI6															
Column 1 (C. comosa)	A11															
	A12															
	A13	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	3-Oct-05	-	
Column 2 (C. comosa)	A21	E8F	1.0	E533R	1.0	12.5	5	5.5	46	3	18.5	10X	2.4	3-Oct-05	+	Complete
	A22															
	A23															
Column 3 (Blank)	A31	E8F	1.0	E533R	1.0	12.5	5.0	5.5	46	3	18.5	10X	2.4	9-Nov-05	+	Complete
	A32	E8F	1.0	E533R	1.0	12.5	0.0	10.5	46	3	18.5	10X	2.4	9-Nov-05	-	
	A33															
Column 4 (E. erythropoda)	A41															
	A42															
	A43															
Column 5 (S. atrovirens)	A51	E8F	1.0	E533R	1.0	12.5	5.0	5.5	46	3	18.5	10X	2.4	9-Nov-05	+	Complete
	A52															
	A53															
Column 6 (S. atrovirens)	A61	E8F	1.0	E533R	1.0	12.5	0.0	12	46	3	18.5	10X	2.4	16-Nov-05	+	
	A62															
	A63															
Column 7 (E. erythropoda)	A71															
	A72															
	A73															
Column 8 (Blank)	A81															
	A82															
	A83															
Column 9 (S. atrovirens)	A91															
	A92															
	A93															
Column 10 (E. erythropoda)	A10-1															
	A10-2															
	A10-3															
Column 11 (Blank)	A11-1															
	A11-2															
	A11-3															
Column 12 (S. atrovirens)	A12-1															
	A12-2															
	A12-3															

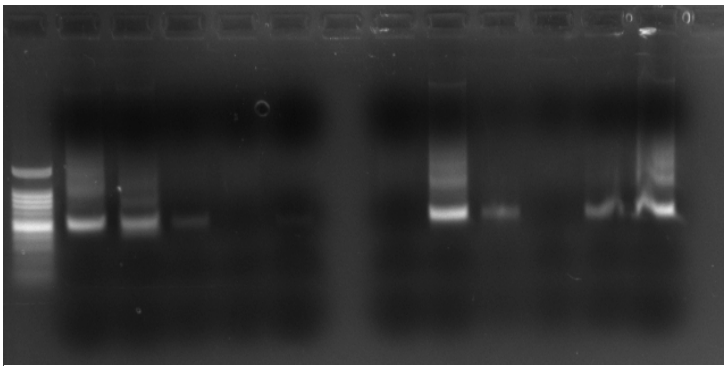
		PCR #6								Gel #6						
		Primers				Master Mix * (µl)	H20 (µl)	Template DNA (µl)	Annealing Temp (°C)	PCR Product (µl)	H20 (µl)	Gel Buffer		PCR #5 (date)	PCR #6 Results	Comments
	Extract #	Forward Primer	Vol. (µl)	Reverse Primer	Vol. (µl)							Conc.	Vol. (µl)			
Soil+Inoculum	SI5															
	SI6															
Column 1 (C. comosa)	A11															
	A12															
	A13	E8F	1.0	E533R	1.0	12.5	0.0	10.5	46	3	18.5	10X	2.4	9-Nov-05	+	Complete
Column 2 (C. comosa)	A21															
	A22															
	A23															
Column 3 (Blank)	A31															
	A32	E8F	1.0	E533R	1.0	12.5	7.5	3	46	3	18.5	10X	2.4	16-Nov-05	+	
	A33															
Column 4 (E. erythropoda)	A41															
	A42															
	A43															
Column 5 (S. atrovirens)	A51															
	A52															
	A53															
Column 6 (S. atrovirens)	A61	E8F	1.0	E533R	1.0	12.5	5.0	5.5	46	3	18.5	10X	2.4	16-Nov-05	+	Complete
	A62															
	A63															
Column 7 (E. erythropoda)	A71															
	A72															
	A73															
Column 8 (Blank)	A81															
	A82															
	A83															
Column 9 (S. atrovirens)	A91															
	A92															
	A93															
Column 10 (E. erythropoda)	A10-1															
	A10-2															
	A10-3															
Column 11 (Blank)	A11-1															
	A11-2															
	A11-3															
Column 12 (S. atrovirens)	A12-1															
	A12-2															
	A12-3															

	Extract #	PCR #7								Gel #7					Pooled Date
		Primers				Master Mix * (µl)	H20 (µl)	Template DNA (µl)	Annealing Temp (°C)	PCR Product (µl)	H20 (µl)	Gel Buffer		PCR #7 (date)	PCR #7 Results	Comments	
		Forward Primer	Vol. (µl)	Reverse Primer	Vol. (µl)							Conc.	Vol. (µl)				
Soil+Inoculum	SI5																3-Nov-05
	SI6																3-Nov-05
Column 1 (C. comosa)	A11																3-Nov-05
	A12																3-Nov-05
	A13																10-Nov-05
Column 2 (C. comosa)	A21																3-Nov-05
	A22																3-Nov-05
	A23																3-Nov-05
Column 3 (Blank)	A31																10-Nov-05
	A32	E8F	1.0	E533R	1.0	12.5	7.5	3	46	3	18.5	10X	2.4	17-Nov-05	+	Complete	17-Nov-05
	A33																3-Nov-05
Column 4 (E. erythropoda)	A41																3-Nov-05
	A42																17-Nov-05
	A43																10-Nov-05
Column 5 (S. atrowirens)	A51																10-Nov-05
	A52																10-Nov-05
	A53																17-Nov-05
Column 6 (S. atrowirens)	A61																17-Nov-05
	A62																3-Nov-05
	A63																3-Nov-05
Column 7 (E. erythropoda)	A71																3-Nov-05
	A72																3-Nov-05
	A73																3-Nov-05
Column 8 (Blank)	A81																3-Nov-05
	A82																3-Nov-05
	A83																10-Nov-05
Column 9 (S. atrowirens)	A91																3-Nov-05
	A92																3-Nov-05
	A93																10-Nov-05
Column 10 (E. erythropoda)	A10-1																10-Nov-05
	A10-2																10-Nov-05
	A10-3																10-Nov-05
Column 11 (Blank)	A11-1																10-Nov-05
	A11-2																3-Nov-05
	A11-3																3-Nov-05
Column 12 (S. atrowirens)	A12-1																3-Nov-05
	A12-2																3-Nov-05
	A12-3																3-Nov-05

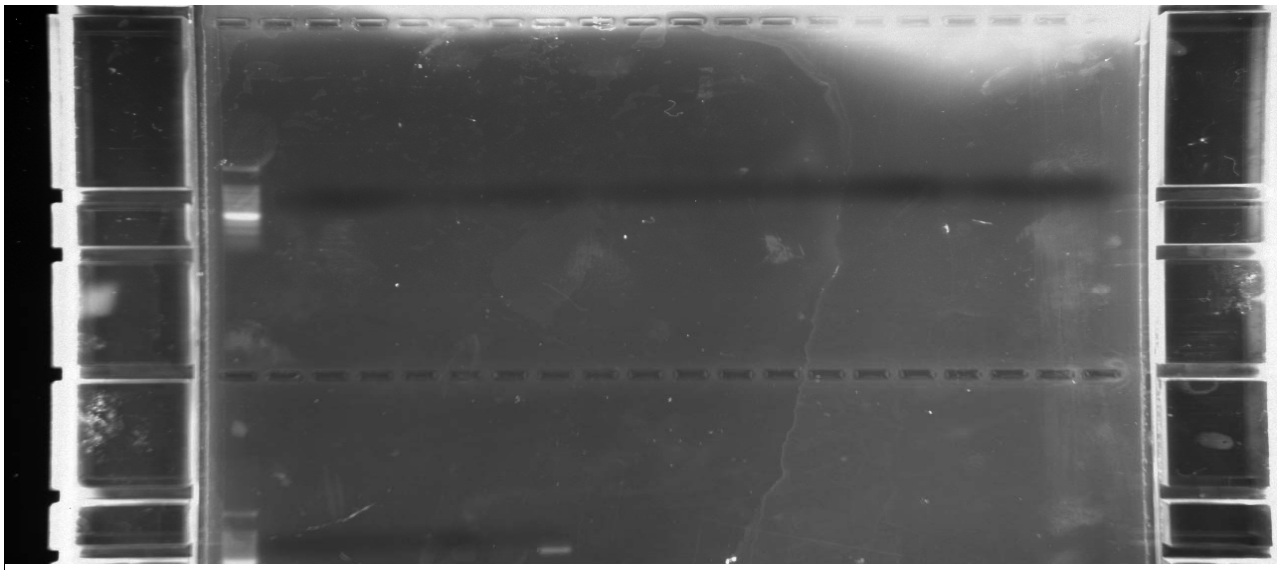
Appendix G. PCR Gels



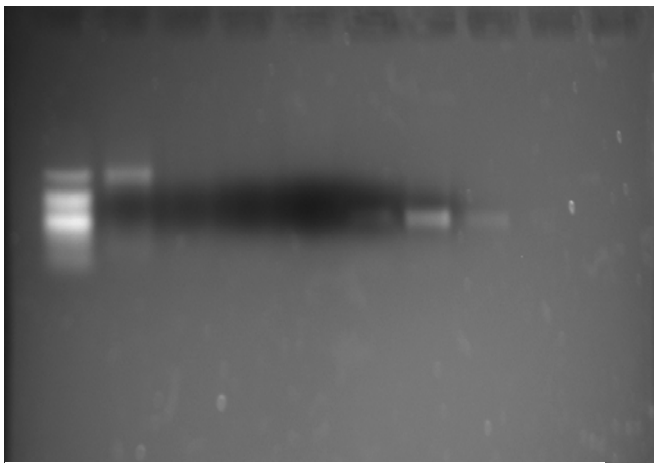
12 Sept Gel: Ladder, A11, A12, A13, A31, A32, A33, A41, A42, A43, A51, A52, A53



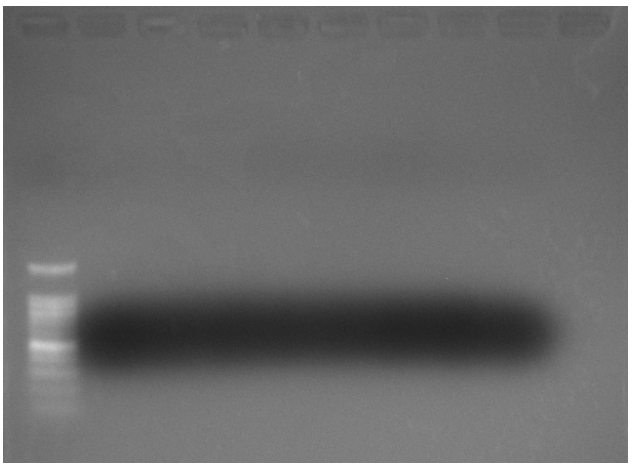
13 Sept Gel: Ladder, A11, A12, A13, A31, A32, None, A41, A42, A43, A51, A52, A53



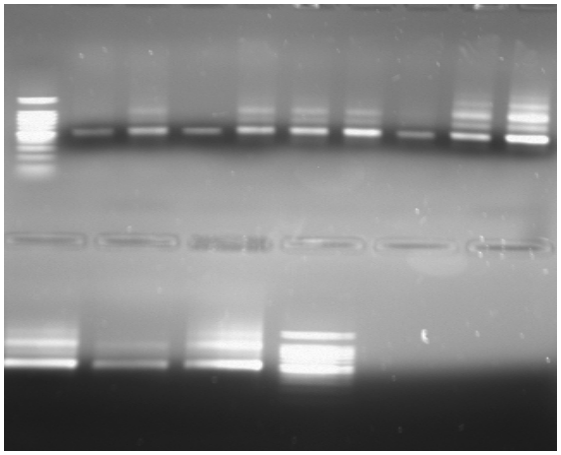
19 Sept Gel: Promega Mastermix. Unamplified samples.
Top Row: Ladder, A21, A22, A23, A33, A61, A62, A63, A91, A72, A73, A81, A82, A83, A71, A92, A93, A10-1, A10-2, A10-3
Bottom Row: Ladder, A11-1, A11-2, A11-3, A12-1, A12-2, A12-3, Blank



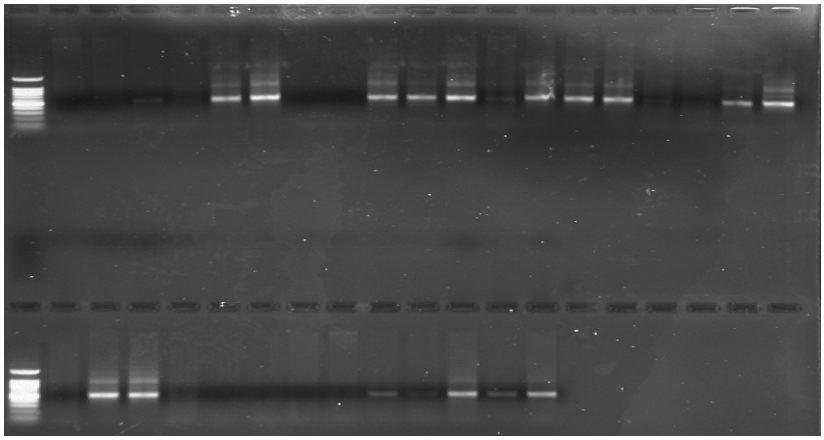
20 Sept Gel: HotStarTaq Mastermix.
Ladder, Lanes 2-5 (G. Joseph's), Blank, A21, A22, A23



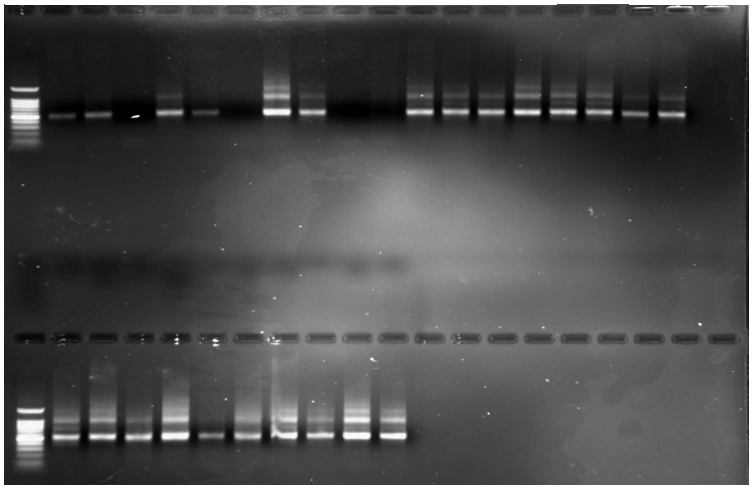
21 Sept Gel: : Promega Mastermix. Multiple unamplified samples.



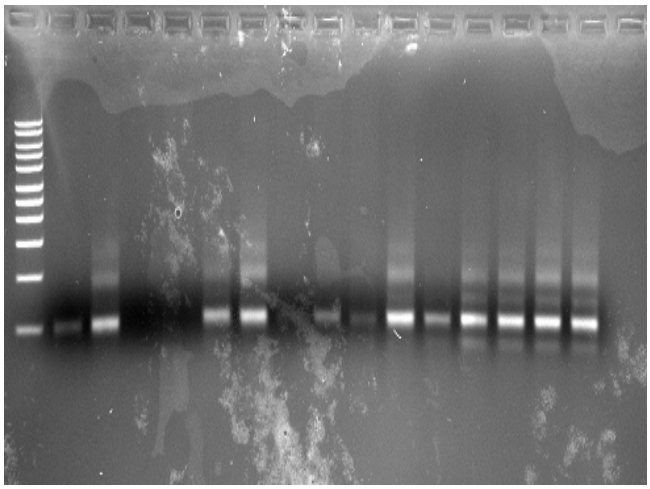
23 Sept Gel: Top: Ladder, SI5, SI6, Blank, A11, A12, A13, A21, A22, A23;
Bottom: A12-1, A12-2, A12-3, Ladder



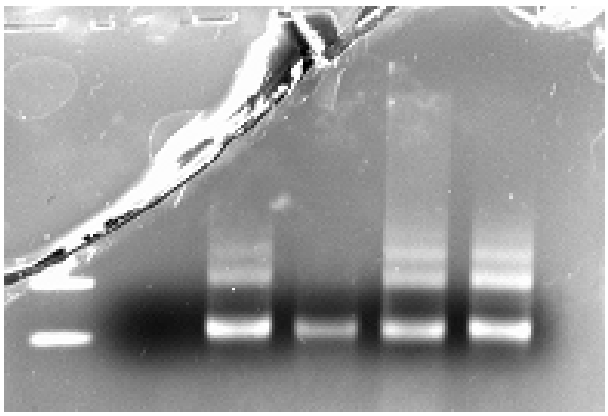
30 Sept Gel: Top: Ladder, SI5, SI6, A13, A21, A22, A23, A31, A32, A33, A41, A51, A61, A62, A63, A71, A72, A73, A81, A82
Bottom: Ladder, A83, A91, A92, A93, A10-1, A10-2, A10-3, A11-1, A11-2, A11-3, A12-1, A12-2, A12-3



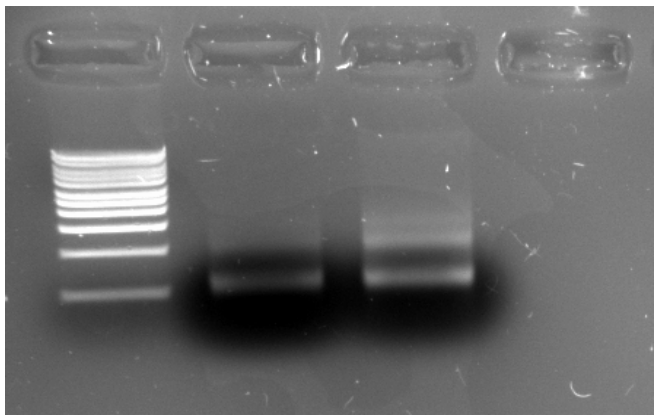
3 Oct Gel: Top: Ladder, SI5, SI6, A13, A21, A31, A32, A33, A41, A51, A61, A62, A63, A71, A72, A73, A81, A82, A83
Bottom: Ladder, A91, A92, A93, A10-1, A10-2, A10-3, A11-1, A11-2, A11-3, A12-2



9 Nov Gel: Ladder, A13, A31, A32, A42, A43, A51, A52, A53, A61, A83, A93, A10-1, A10-2, A10-3, A11-1



16 Nov Gel: Ladder, A32 (12 ul DNA), A32 (3 ul DNA), A42 (5.5 ul DNA), A61 (12 ul DNA), A61 (5.5 ul DNA),



17 Nov Gel: Ladder, A32, A53

Appendix H. Invitrogen Topo Cloning Protocol (Invitrogen Corporation, 2004)

Setting Up the TOPO® Cloning Reaction

The table below describes how to set up your TOPO® Cloning reaction (6 µl) for eventual transformation into chemically competent TOP10 *E. coli*.

Note: The red color of the TOPO® vector solution is normal and is used to visualize the solution.

Reagent*	**Chemically Competent *E. coli***
Fresh PCR product	0.5 to 4 µl
Salt Solution	1 µl
Sterile Water	add to a total volume of 5 µl
TOPO® vector	1 µl
Final Volume	**6 µl**

* Store all reagents at -20°C when finished. Salt solutions and water can be stored at room temperature or +4°C.

Performing the TOPO® Cloning Reaction

1. Mix reaction gently and incubate for **5 minutes** at room temperature (22-23°C).

   Note: For most applications, 5 minutes will yield plenty of colonies for analysis. Depending on your needs, the length of the TOPO® Cloning reaction can be varied from 30 seconds to 30 minutes. For routine subcloning of PCR products, 30 seconds may be sufficient. For large PCR products (> 1 kb) or if you are TOPO® Cloning a pool of PCR products, increasing the reaction time will yield more colonies.

2. Place the reaction on ice and proceed to **General Guidelines for Transforming Competent Cells**.

   Note: You may store the TOPO® Cloning reaction at -20°C overnight.

Transforming One Shot® TOP10 Competent Cells

Introduction

Protocols to transform One Shot® TOP10 competent *E. coli* are provided below.

Materials Supplied by the User

In addition to general microbiological supplies (*e.g.* plates, spreaders), you will need the following reagents and equipment.

- TOPO® Cloning reaction from Performing the TOPO® Cloning Reaction, Step 2.
- S.O.C. medium (included with the kit)
- LB plates containing 50 µg/ml kanamycin
- 40 mg/ml X-gal in dimethylformamide (DMF)
- 42°C water bath or an electroporator and 0.1 or 0.2 cm cuvettes
- 37°C shaking and non-shaking incubator

Preparation for Transformation

For each transformation, you will need one vial of competent cells and two selective plates.

- Equilibrate a water bath to 42°C (for chemical transformation).
- Warm the vial of S.O.C. medium from Box 2 to room temperature.
- Warm selective plates at 37°C for 30 minutes (see Important note below).
- Spread 40 µl of 40 mg/ml X-gal on each LB plate and incubate at 37°C until ready for use.
- Thaw **on ice** 1 vial of One Shot® cells for each transformation.

One Shot® Chemical Transformation Protocol

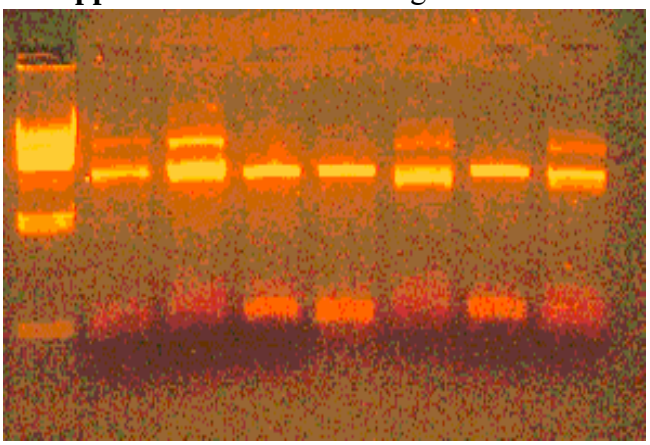
1. Add 2 µl of the TOPO® Cloning reaction from Performing the TOPO® Cloning Reaction, Step 2, into a vial of One Shot® Chemically Competent *E. coli* and mix gently. **Do not mix by pipetting up and down**.
2. Incubate on ice for 5 to 30 minutes.
Note: Longer incubations on ice do not seem to have any affect on transformation efficiency. The length of the incubation is at the user's discretion.
3. Heat-shock the cells for 30 seconds at 42°C without shaking.
4. Immediately transfer the tubes to ice.
5. Add 250 µl of room temperature S.O.C. medium.
6. Cap the tube tightly and shake the tube horizontally (200 rpm) at 37°C for 1 hour.
7. Spread 10-50 µl from each transformation on a prewarmed selective plate and incubate overnight at 37°C. To ensure even spreading of small volumes, add 20 µl of S.O.C. medium We recommend that you plate two different volumes to ensure that at least one plate will have well-spaced colonies.
8. An efficient TOPO® Cloning reaction should produce several hundred colonies. Pick ~10 white or light blue colonies for analysis (see Analyzing Positive Clones, page 12). Do not pick dark blue colonies.

Analyzing Transformants

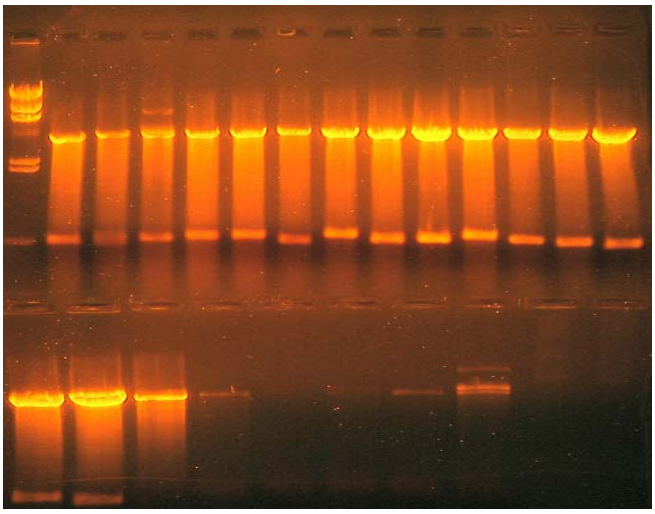
Analyzing Positive Clones

1. Take the 10 white or light blue colonies and culture them overnight in LB medium containing 50 µg/ml ampicillin or 50 µg/ml kanamycin.
2. Isolate plasmid DNA using your method of choice.
3. Analyze the plasmids by restriction analysis to confirm the presence and correct orientation of the insert. Use a restriction enzyme or a combination of enzymes that cut once in the vector and once in the insert.

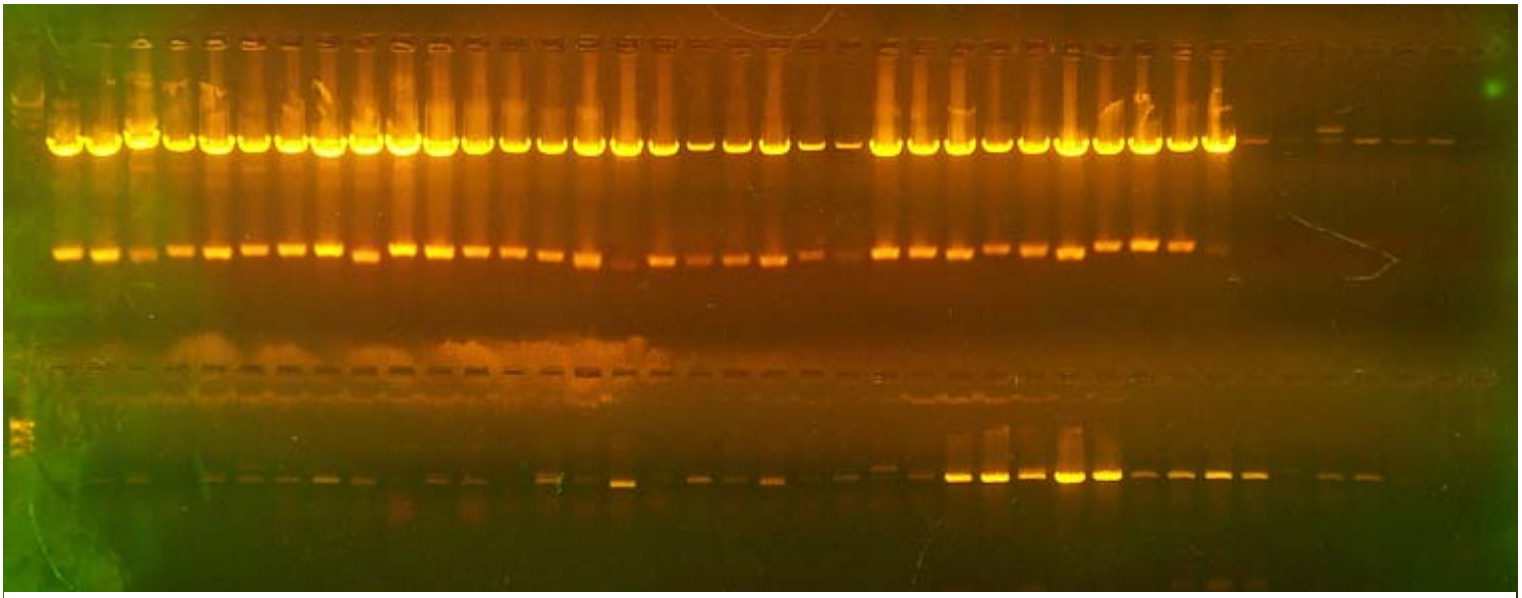
Appendix I. Restriction Digestion Gels



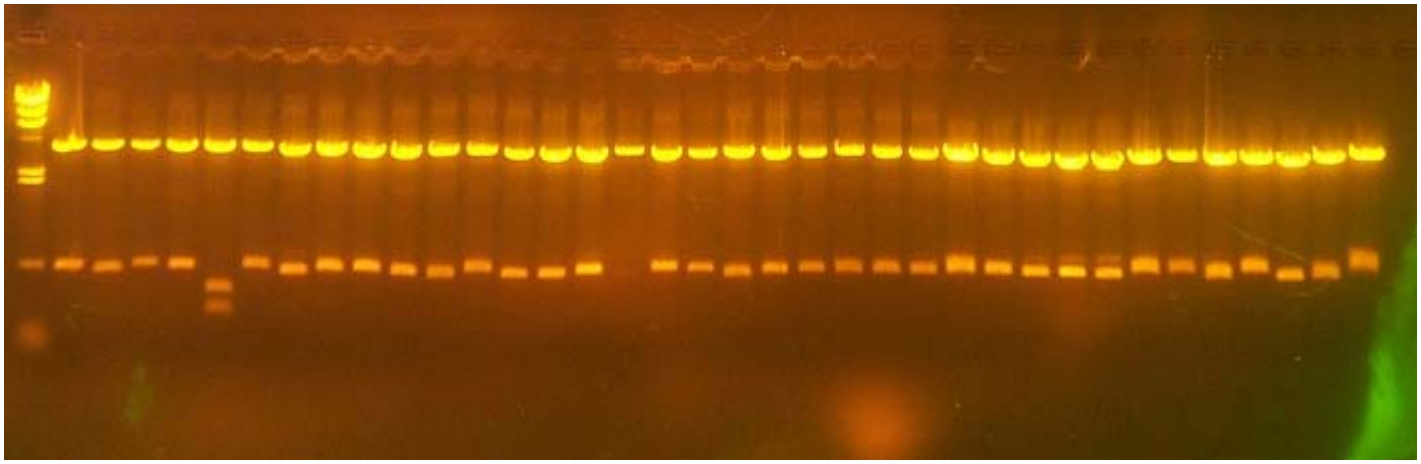
29 Nov Gel: λ Ladder; A11: 1.1, 1.2, 2.1, 3.5; NC A11: 1, 2, 3



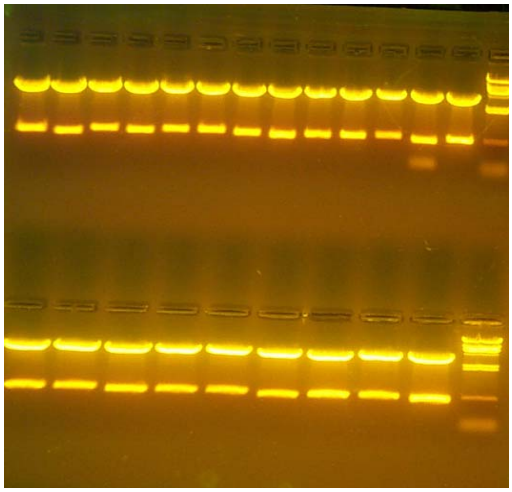
1 Dec Gel: Top: λ Ladder; A71: 1.1, 1.2, 1.3, 1.4, 1.5, 2.1, 2.2, 2.3, 2.4, 2.5, 3.1, 3.2, 3.3
Bottom: A71: 3.4, 3.5, Negative Control (NC); A11: 2.1.4, 2.1.5, 1.2.2, 2.3.2, 1.3.2



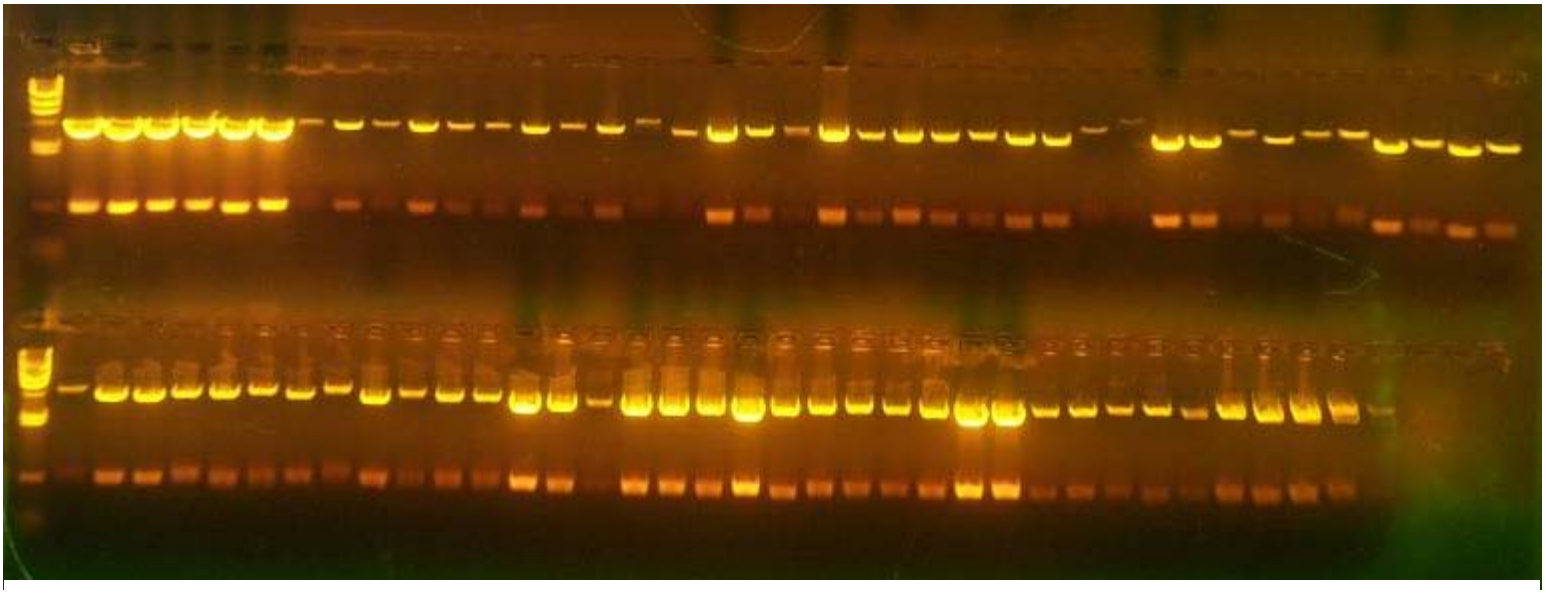
2 Dec Gel: Top: λ Ladder; A72: 1.1, 1.2, 1.3, 1.4, 1.5, 2.1, 2.2, 2.3, 2.4, 2.5, 3.1, 3.2, 3.3, 3.4, 3.5, NC; A73: : 1.1, 1.2, 1.3, 1.4, 1.5, 2.1, 2.2, 2.3, 2.4, 2.5, 3.1, 3.2, 3.3, 3.4, 3.5, NC; SI5: 1.1, 1.2, 1.3, 1.4, 1.5, 2.1, 2.2
Bottom: None; SI5: 2.3, 2.4, 2.5, 3.1, 3.2, 3.3, 3.4, 3.5; SI6: 1.1, 1.2, 1.3, 1.4, 1.5, 2.1, 2.2, 2.3, 2.4, 2.5, 3.1, 3.2, 3.3, 3.4, 3.5; NC SI5: 1,2,3; NC SI6: 1, 2, 3; A11: 1.1, 3.2, 1.5, 2.2, 3.2



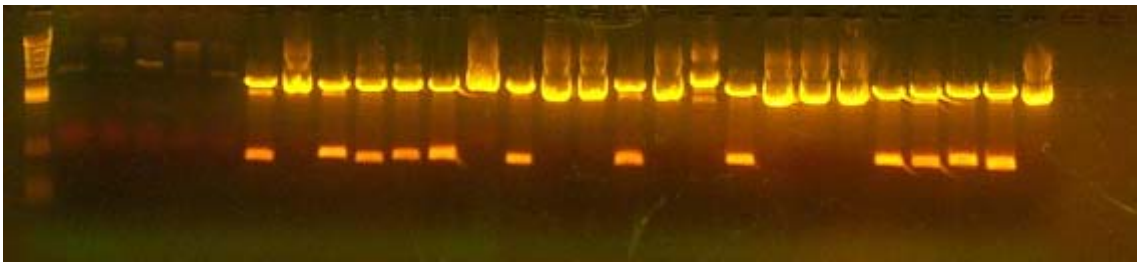
6 Dec Gel: λ Ladder; A81: 1.1, 1.2, 1.3, 1.4, 1.5, 2.1, 2.2, 2.3, 2.4, 2.5, 3.1, 3.2, 3.3, 3.4, 3.5, NC; A82: 1.1, 1.2, 1.4, 1.3, 1.5, 2.1, 2.2, 2.3, 2.4, 2.5, 3.1, 3.2, 3.3, 3.4, 3.5; A83: 1.1, 1.2, 1.3, 1.4, 1.5



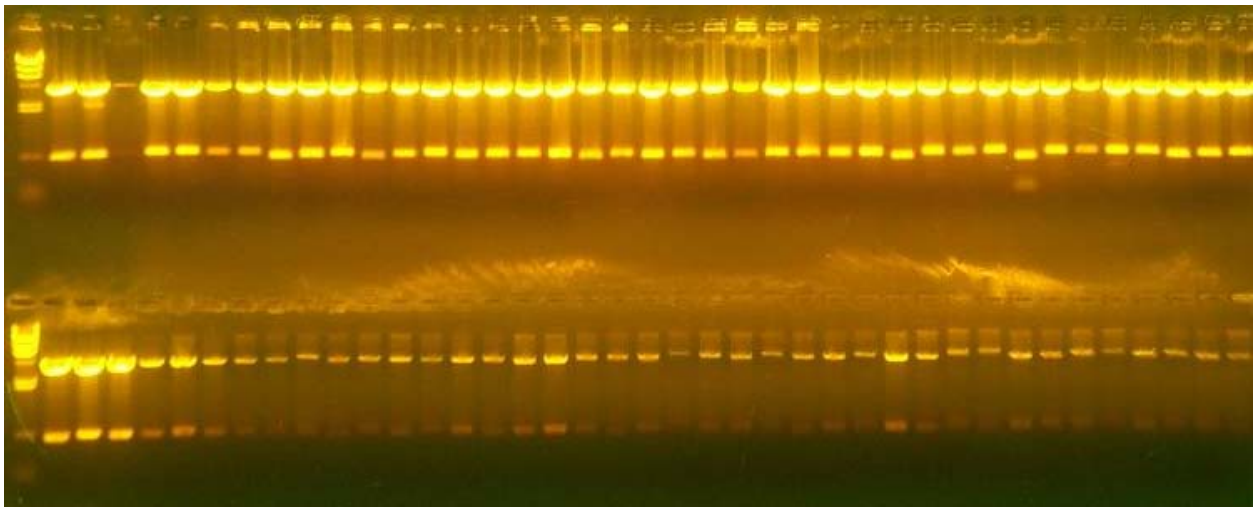
9 Dec Gel: Top: A91: 1.1, 1.2, 1.3, 1.4, 1.5, 2.1, 2.2, 2.3, 2.4, 2.5, 3.1, 3.2, 3.3; λ Ladder
Bottom: A91.3.5; A92: 1.1, 1.2, 1.3, 1.5, 2.1, 2.2, 2.3, 2.4; λ Ladder



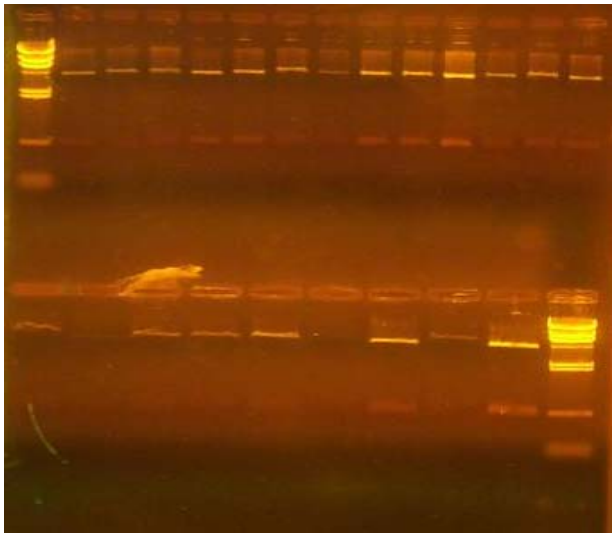
9 Dec Gel: Top: λ Ladder; A92: 2.5, 3.1, 3.2, 3.3, 3.4, 3.5; A83: 2.1, 2.2, 2.3, 2.4, 2.5, 3.1, 3.2, 3.3, 3.4, 3.5, NC; SI5: 1.5, 2.1, 2.2, 2.3, 2.4, 2.5, 3.1, 3.2, 3.3, 3.4, 1.3, 1.2, 1.1; SI6: 3.5, 3.4, 3.3, 3.2, 3.1, 2.5, 2.4; A12.3.2; SI5.3.5
Bottom: λ Ladder; SI5.1.4; A12: 3.3, 3.4, 3.5; SI6: 1.1, 1.2, 1.3, 1.4, 1.5, 2.1, 2.2, 2.3; A12: 3.1, 2.5, 2.4, 2.3, 2.2, 2.1, 1.5, 1.4, 1.3, 1.2, 1.1; A13.1.1; A93.1.2, A93.1.1; A13: 3.1, 2.5, 2.4, 2.3, 2.2, 2.1, 1.5, 1.4, 1.3, 1.2



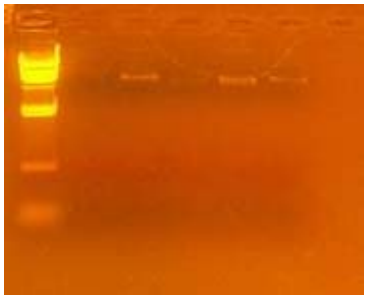
12 Dec Gel: λ Ladder; A11: 3.2, 1.4, 1.5, 2.2, 2.3.2, A10-1: 2.1, 2.2, 2.3, 2.4, 2.5, 1.1, 1.2, 1.3, 1.4; A93: 3.1, 3.2, 3.3, 3.4, 3.5, 2.1, 2.2, 2.3, 2.4, 2.5, 1.3, 1.4, 1.5



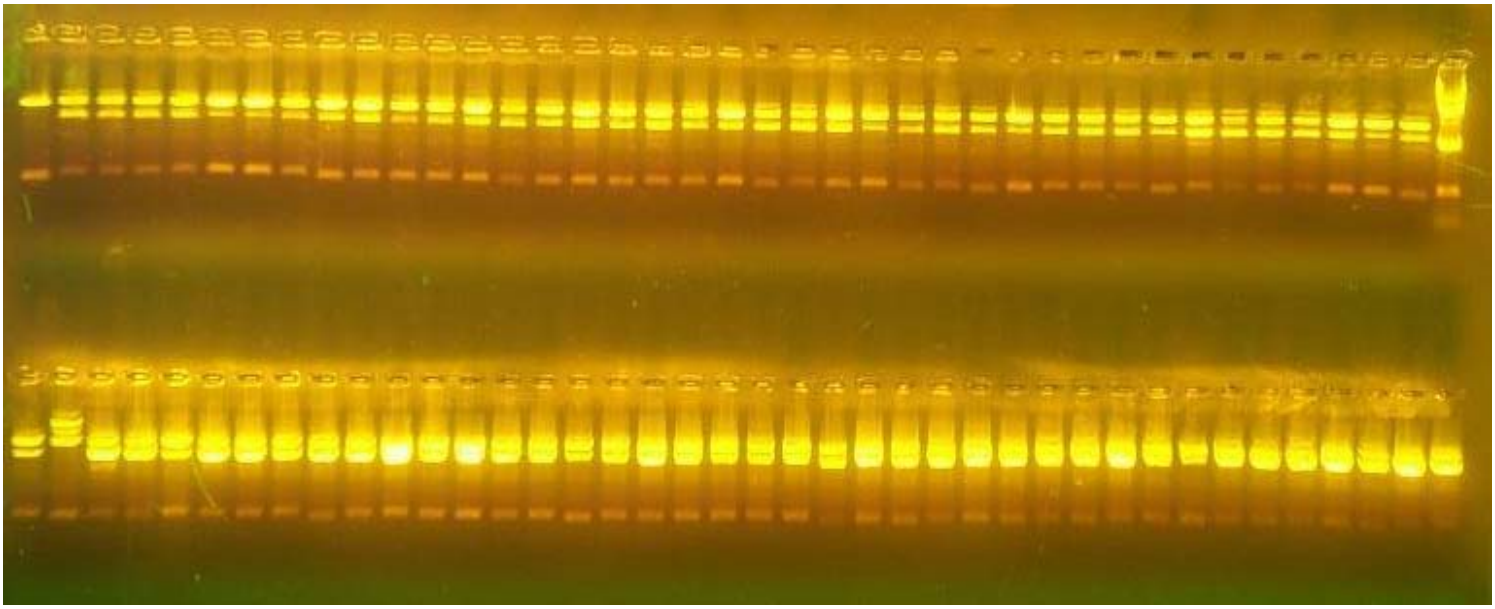
22 Dec Gel: Top: λ Ladder; A93: 1.5, 2.3; A10-1: 3.1, 3.2, 3.3, 3.4, 3.5; A13: 3.1, 3.2, 3.3, 3.4, 3.5; A10-2: 1.1, 1.2, 1.3, 1.4, 1.5, 2.1, 2.2, 2.3, 2.4, 2.5, 3.1, 3.2, 3.3, 3.4, 3.5; A10-3: 1.1, 1.2, 1.3, 1.4, 1.5, 2.1, 2.2, 2.3, 2.4, 2.5, 3.1, 3.2
Bottom: λ Ladder; A10-3: 3.3, 3.4, 3.5; A21: 1.1, 1.2, 1.3, 1.4, 2.1, 2.2, 2.3, 2.4, 2.5, 3.1, 3.3, 3.4, 3.5; A22: 1.1, 1.2, 1.3, 1.4, 1.5, 2.1, 2.2, 2.3, 2.4, 2.5, 3.1, 3.2, 3.3, 3.4, 3.5; A23: 1.1, 1.2, 1.3, 1.4, 1.5, 2.1; A31: 1.1, 1.2



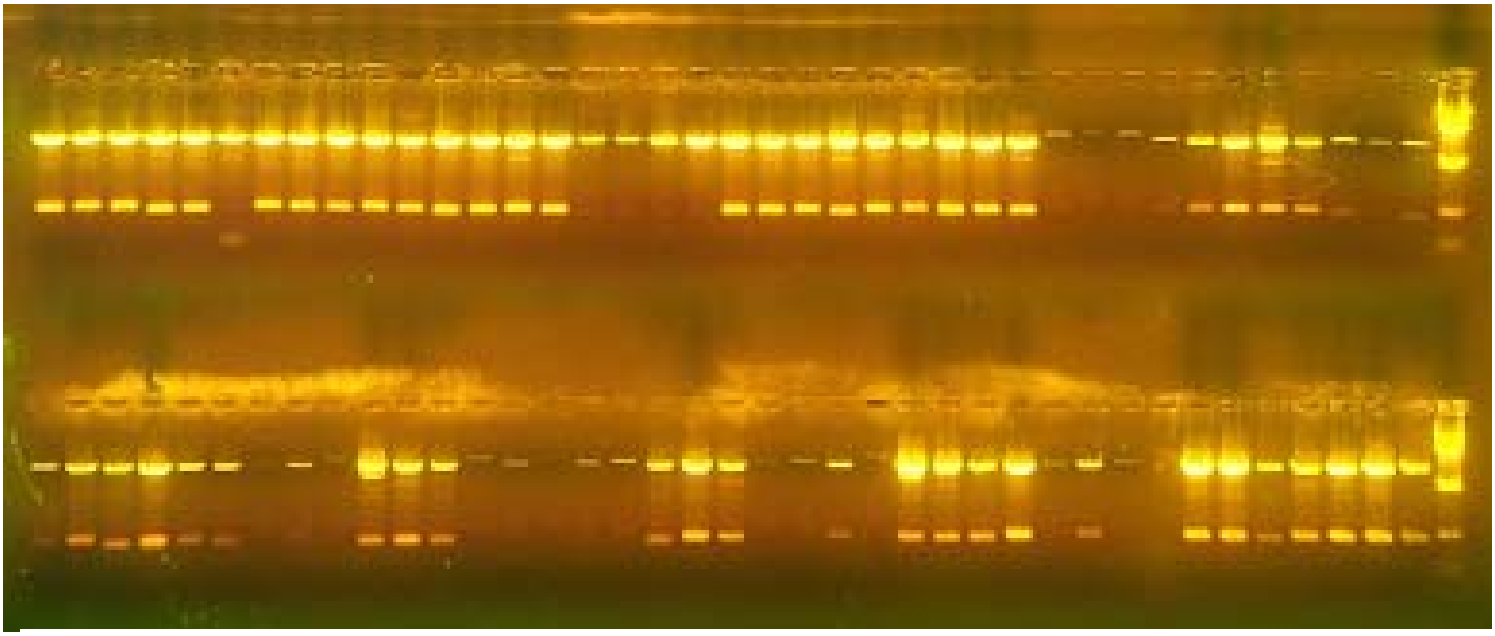
22 Dec Gel: Top: λ Ladder; A31: 1.3, 1.4, 1.5, 2.1, 2.2, 2.3, 2.4, 2.5, 3.1, 3.3, 3.4, 3.5;
Bottom: A32: 1.1, 1.2, 1.3, 1.4, 1.5, 2.1, 2.2, 2.3, 2.4; λ Ladder



22 Dec Gel: λ Ladder; A32: 2.5, 3.1, 3.2, 3.3, 3.5



20 Jan Gel: Top: A32.2: 1.1, 1.2, 1.3, 1.4, 1.5, 2.1, 2.2, 2.3, 2.4, 2.5, 3.1, 3.2, 3.3, 3.4, 3.5; A62: 1.1, 1.2, 1.3, 1.4, 1.5, 2.1, 2.2, 2.3, 2.4, 2.5, 3.1, 3.2, 3.3, 3.4, 3.5; A63: 1.1, 1.2, 1.3, 1.4, 1.5, 2.1, 2.2, 2.3, 2.4; λ Ladder
Bottom: A63: 2.5, 3.1, 3.2, 3.3, 3.4, 3.5; A11-2: 1.1, 1.2, 1.3, 1.4, 1.5, 2.1, 2.2, 2.3, 2.4, 2.5, 3.1, 3.2, 3.3, 3.4, 3.5; A12-2: 1.1, 1.2, 1.3, 1.4, 1.5, 3.1, 3.2, 3.3, 3.4, 3.5; A23: 2.2, 2.3, 2.4, 2.5, 3.1, 3.2, 3.3, 3.4, 3.5



20 Jan Gel: Top: A11.2: 1.1, 1.2, 1.3, 1.4, 1.5, 2.1, 2.2, 2.3, 2.4, 2.5, 3.1, 3.2, 3.3, 3.4, 3.5; Blank: 1,2,3,4; A13: 1.6, 1.7, 1.8, 2.6, 2.7, 2.8, 3.6, 3.7, 3.8; A41: 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 2.1, 2.2, 2.3; λ Ladder
Bottom: A41: 2.5, 2.6, 2.7, 2.8, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8; A42: 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 2.1, 2.2, 2.3, 2.4; A10-2.2.6; A42: 2.6, 2.7, 2.8, 3.1, 3.2, 3.3; None; A10-2.2.7; A42: 3.6, 3.7, 3.8; A10-1: 2.6, 2.7; A41.2.4; λ Ladder

Appendix J. Isolated Plasmid DNA Concentrations

Restriction Digested	Insert?	Sample ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Restriction Digested	Insert?	Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230
√		Blank1	1/3/2006	2:43 PM	61.54	1.231	0.615	2	2.13											
√		Blank2	1/3/2006	2:44 PM	59.24	1.185	0.604	1.96	2.24											
√		Blank3NC	1/3/2006	2:45 PM	136.15	2.723	1.377	1.98	2.21											
√		Blank4NC	1/3/2006	2:46 PM	175.24	3.505	1.819	1.93	1.87											
√	√	SI5.1.1	12/13/2005	3:03 PM	87.29	1.746	0.938	1.86	0.6											
√		SI5.1.2	12/13/2005	3:04 PM	3.64	0.073	0.05	1.44	0.05	√	√	SI5.1.2	Default	1/6/2006	3:41 PM	35.68	0.714	0.404	1.77	1.22
√		SI5.1.3	12/13/2005	3:05 PM	14.27	0.285	0.158	1.81	0.59	√	√	SI5.1.3	Default	1/6/2006	3:42 PM	80.26	1.605	0.923	1.74	1.07
√	√	SI5.1.4	12/13/2005	3:06 PM	15.23	0.305	0.188	1.62	0.1	√	√	SI5.1.4	Default	1/6/2006	3:43 PM	57.23	1.145	0.597	1.92	2.08
√	√	SI5.1.5	12/13/2005	3:07 PM	93.93	1.879	0.983	1.91	0.76											
√	√	SI5.2.1	12/13/2005	3:08 PM	30.15	0.603	0.318	1.9	0.73											
√		SI5.2.2	12/13/2005	3:09 PM	30.04	0.601	0.35	1.72	0.11			SI5.2.2	Default	1/6/2006	3:44 PM	19.15	0.383	0.224	1.71	1.78
√	√	SI5.2.3	12/13/2005	3:10 PM	175.26	3.505	1.889	1.86	0.39			SI5.2.3	Default	1/6/2006	3:45 PM	12.26	0.245	0.146	1.68	1.59
√	√	SI5.2.4	12/13/2005	3:11 PM	19.44	0.389	0.206	1.88	-12.22	√	√	SI5.2.4	Default	1/6/2006	3:46 PM	39.84	0.797	0.476	1.68	0.77
√	√	SI5.2.5	12/13/2005	3:12 PM	83.17	1.663	0.891	1.87	0.53			SI5.2.5	Default	1/6/2006	3:47 PM	44.26	0.885	0.543	1.63	0.74
√	√	SI5.3.1	12/13/2005	3:13 PM	21.45	0.429	0.231	1.86	0.29	√	√	SI5.3.1	Default	1/6/2006	3:48 PM	75.91	1.518	0.798	1.9	2.11
√	√	SI5.3.2	12/13/2005	3:14 PM	23.17	0.463	0.285	1.63	0.15	√	√	SI5.3.2	Default	1/6/2006	3:49 PM	64.66	1.293	0.697	1.85	2.06
√	√	SI5.3.3	12/13/2005	3:16 PM	37.41	0.748	0.414	1.81	0.82	√		SI5.1NC	Default	1/6/2006	3:50 PM	57.77	1.155	0.656	1.76	1.17
√	√	SI5.3.4	12/13/2005	3:17 PM	75.28	1.506	0.805	1.87	1.53	√		SI5.2NC	Default	1/6/2006	3:51 PM	87.15	1.743	0.999	1.75	0.99
√	√	SI5.3.5	12/13/2005	3:18 PM	36.51	0.73	0.377	1.94	1.14	√		SI5.3NC	Default	1/6/2006	3:52 PM	73.15	1.463	0.787	1.86	1.99
√	√	SI6.1.1	12/13/2005	3:19 PM	122.27	2.445	1.308	1.87	1.31											
√	√	SI6.1.2	12/13/2005	3:22 PM	38.9	0.778	0.418	1.86	0.3	√		SI6.1.2	Default	1/6/2006	3:53 PM	265.1	5.302	2.859	1.85	1.56
√	√	SI6.1.3	12/13/2005	3:30 PM	35.68	0.714	0.326	2.19	1.92	√		SI6.1.3	Default	1/6/2006	3:53 PM	71.69	1.434	0.799	1.79	1.37
√	√	SI6.1.4	12/13/2005	3:33 PM	53.7	1.074	0.563	1.91	0.19	√	√	SI6.1.4	Default	1/6/2006	3:54 PM	145.48	2.91	1.558	1.87	1.7
√	√	SI6.1.5	12/13/2005	3:34 PM	39.02	0.78	0.382	2.04	0.72											
√	√	SI6.2.1	12/13/2005	3:35 PM	53.18	1.064	0.593	1.79	0.23			SI6.2.1	Default	1/6/2006	3:56 PM	15.21	0.304	0.2	1.52	14.9
√	√	SI6.2.2	12/13/2005	3:36 PM	17.58	0.352	0.213	1.65	0.19	√	√	SI6.2.2	Default	1/6/2006	3:56 PM	51.07	1.021	0.583	1.75	1.23
√	√	SI6.2.3	12/13/2005	3:37 PM	19.43	0.389	0.19	2.05	1.15	√	√	SI6.2.3	Default	1/6/2006	3:57 PM	73.18	1.464	0.869	1.68	0.85
√	√	SI6.2.4	12/13/2005	3:38 PM	67.05	1.341	0.812	1.65	0.18			SI6.2.4	Default	1/6/2006	3:58 PM	16.89	0.338	0.213	1.58	1.07
√	√	SI6.2.5	12/13/2005	3:39 PM	85.21	1.704	0.888	1.92	0.98											
√	√	SI6.3.1	12/13/2005	3:39 PM	85.76	1.715	1.1	1.56	0.2	√	√	SI6.3.1	Default	1/6/2006	3:59 PM	158.94	3.179	1.727	1.84	1.58
√		SI6.3.2	12/13/2005	3:40 PM	43.82	0.876	0.518	1.69	0.16			SI6.3.2	Default	1/6/2006	3:59 PM	21.61	0.432	0.251	1.73	2.12
√	√	SI6.3.3	12/13/2005	3:42 PM	10.3	0.206	0.12	1.72	1.73	√	√	SI6.3.3	Default	1/6/2006	4:00 PM	87.16	1.743	0.988	1.77	1.16
√		SI6.3.4	12/13/2005	3:43 PM	50.78	1.016	0.594	1.71	0.21	√	√	SI6.3.4	Default	1/6/2006	4:01 PM	81.85	1.637	0.911	1.8	1.24
√	√	SI6.3.5	12/13/2005	3:44 PM	66.55	1.331	0.697	1.91	3.05	√	√	SI6.3.5	Default	1/6/2006	4:02 PM	75.16	1.503	0.854	1.76	1.33
√	√	A11.1.1	1/6/2006	12:21 PM	75.59	1.512	0.732	2.06	1.38											
√	√	A11.1.2	1/6/2006	12:24 PM	72.84	1.457	0.682	2.14	1.6											
√	√	A11.2.1	1/6/2006	12:29 PM	82.39	1.648	0.797	2.07	1.31											
√		A11.3.1	1/6/2006	12:38 PM	102.66	2.053	1.009	2.04	1.66											
√		A11.1.1.1	1/9/2006	5:41 PM	42.72	0.854	0.53	1.61	0.74											
√		A11.1.2.4	1/9/2006	5:42 PM	21.77	0.435	0.245	1.78	0.85											
√	√	A11.2.1.1	1/13/2006	2:20 PM	407.35	8.147	4.219	1.93	2											
√	√	A11.2.1.2	1/13/2006	2:21 PM	316.86	6.337	3.285	1.93	1.92											
√	√	A11.2.1.3	1/13/2006	2:22 PM	425.29	8.506	4.408	1.93	2.11											
√	√	A11.2.1.4	1/13/2006	2:23 PM	440.81	8.816	4.6	1.92	1.97											
√	√	A11.2.1.5	1/13/2006	2:24 PM	420.29	8.406	4.37	1.92	1.97											
√	√	A11.2.2.1	1/13/2006	2:24 PM	316	6.32	3.291	1.92	1.85											
√	√	A11.2.2.2	1/13/2006	2:25 PM	431.26	8.625	4.461	1.93	1.91											
√	√	A11.2.2.3	1/13/2006	2:26 PM	341.45	6.829	3.54	1.93	1.91											
√	√	A11.2.2.4	1/13/2006	2:27 PM	371.94	7.439	3.858	1.93	2											
√	√	A11.2.2.5	1/13/2006	2:28 PM	369.09	7.382	3.876	1.9	1.83											
√	√	A11.2.3.1	1/13/2006	2:28 PM	273.69	5.474	2.851	1.92	1.86											
√	√	A11.2.3.2	1/13/2006	2:29 PM	388.05	7.761	4.044	1.92	1.8											
√	√	A11.2.3.3	1/13/2006	2:30 PM	386.12	7.722	3.914	1.97	2.12											
√	√	A11.2.3.4	1/13/2006	2:31 PM	414.68	8.294	4.256	1.95	2.09											
√	√	A11.2.3.5	1/13/2006	2:32 PM	354.38	7.088	3.672	1.93	1.9											
√	√	A12.1.1	12/13/2005	3:45 PM	69.15	1.383	0.815	1.7	0.41											
√	√	A12.1.2	12/13/2005	3:46 PM	39.4	0.788	0.417	1.89	0.2											
√	√	A12.1.3	12/13/2005	3:47 PM	160.06	3.201	1.651	1.94	1.46											
√	√	A12.1.4	12/13/2005	3:48 PM	125.1	2.502	1.322	1.89	0.59			Sequence								
√	√	A12.1.5	12/13/2005	3:49 PM	120.59	2.412	1.221	1.97	2.66											
√	√	A12.2.1	12/15/2005	2:07 PM	103.85	2.077	1.07	1.94	2.18											
√	√	A12.2.2	12/15/2005	2:08 PM	112.03	2.241	1.141	1.96	1.75											
√	√	A12.2.3	12/15/2005	2:10 PM	120.66	2.413	1.241	1.94	1.17											
√		A12.2.4	12/15/2005	2:11 PM	18.33	0.367	0.191	1.92	1.76											
√	√	A12.2.5	12/15/2005	2:12 PM	142.75	2.855	1.462	1.95	0.72											
√	√	A12.3.1	12/15/2005	2:14 PM	244.62	4.892	2.54	1.93	0.73											
√	√	A12.3.2	12/15/2005	2:15 PM	106.27	2.125	1.146	1.85	0.45											
√	√	A12.3.3	12/15/2005	2:17 PM	151.52	3.03	1.693	1.79	0.48											
√	√	A12.3.4	12/15/2005	2:19 PM	82.62	1.652	0.86	1.92	1.48											
√	√	A12.3.5	12/15/2005	2:20 PM	30.62	0.612	0.337	1.82	0.83											
√	√	A13.1.1	12/15/2005	2:21 PM	78.21	1.564	0.807	1.94	2.18											
√		A13.1.2	12/15/2005	2:22 PM	30	0.6	0.299	2.01	1.12											
√	√	A13.1.3	12/15/2005	2:24 PM	50.06	1.001	0.54	1.85	0.59											
√	√	A13.1.4	12/15/2005	2:25 PM	119.72	2.394	1.309	1.83	0.35											
√	√	A13.1.5	12/15/2005	2:26 PM	80.04	1.601	0.829	1.93	0.84											
√	√	A13.1.6	1/19/2006	2:08 PM	462.23	9.245	4.749	1.95	2.22											
√	√	A13.1.7	1/19/2006	2:09 PM	406.51	8.13	4.18	1.94	2.21											
√	√	A13.1.8	1/19/2006	2:10 PM	282.06	5.641	2.862	1.97	2.58											
√	√	A13.2.1	12/15/2005	2:27 PM	61.6	1.232	0.677	1.82	0.39											
√	√	A13.2.2	12/15/2005	2:35 PM	21.57	0.431	0.223	1.94	0.79											
√	√	A13.2.3	12/15/2005	2:35 PM	45.88	0.918	0.498	1.84	0.85											
√	√	A13.2.4	12/15/2005	2:36 PM	29.02	0.58	0.3	1.94	0.78											
√	√	A13.2.5	12/15/2005	2:37 PM	41.5	0.83	0.447	1.86	0.87											
√	√	A13.2.6	1/19/2006	2:11 PM	309.86	6.197	3.176	1.95	2.36											
√	√	A13.2.7	1/19/2006	2:11 PM	391.44	7.829	4.025	1.94	2.19											
√	√	A13.2.8	1/19/2006	2:12 PM	255.62	5.112	2.623	1.95	2.21											
√	√	A13.3.1	12/15/2005	2:38 PM	95.15	1.903	0.918	2.07	1.54											
√	√	A13.2.3.1	12/30/2005	11:33 AM	571.53	11.431	5.973	1.91	2.12											
√	√	A13.3.2	12/30/2005	11:34 AM	534.78	10.696	5.568	1.92	2.13											
√	√	A13.3.3	12/30/2005	11:35 AM	481.49	9.63	4.965	1.94	2.13											
√	√	A13.3.4	12/30/2005	11:35 AM	380.08	7.602	3.92	1.94	2.11											
√	√	A13.3.5	12/30/2005	11:36 AM	496.98	9.94	5.142	1.93	2.13											
√	√	A13.3.6	1/19/2006	2:13 PM	638.58	12.772	6.541	1.95	2.21											
√	√	A13.3.7	1/19/2006	2:14 PM	425.69	8.514	4.371	1.95	2.21											
√	√	A13.3.8	1/19/2006	2:15 PM	296.12	5.922	3.016	1.96	2.2											
√	√	A21.1.1	1/23/2006	2:11 PM	27.69	0.554	0.295	1.88	0.89			A21.2.1.1	Default	2/3/2006	1:43 PM	171.17	3.423	1.742	1.96	2.2
√	√	A21.1.1	1/23/2006	2:13 PM	35.05	0.701	0.375	1.87	0.97			A21.2.1.2	Default	2/3/2006	1:44 PM	156.78	3.136	1.626	1.93	2.22
√	√	A21.1.2	1/23/2006	2:14 PM	32.07	0.641	0.338	1.9	1.26			A21.2.1.3	Default	2/3/2006	1:45 PM	290.82	5.816	2.994	1.94	2.23
√	√	A21.1.3	1/23/2006	2:15 PM	20.36	0.407	0.219	1.86	0.86			A21.2.1.4	Default	2/3/2006	1:46 PM	363.31	7.266	3.745	1.94	2.21
√	√	A21.1.4	1/23/2006	2:16 PM	7.72	0.154	0.104	1.49	0.56			A21.2.1.5	Default	2/3/2006	1:47 PM	322.07	6.441	3.319	1.94	2.21
√	√	A21.2.1	1/23/2006	2:16 PM	7.38	0.148	0.088	1.68	0.37			A21.2.2.1	Default	2/3/2006	1:48 PM	258.99	5.18	2.673	1.94	2.18
√	√	A21.2.2	1/23/2006	2:36 PM	12.27	0.245	0.137	1.79	0.53			A21.2.2.2	Default	2/3/2006	1:49 PM	257.07	5.141	2.655	1.94	2.22
√	√	A21.2.3	1/23/2006	2:37 PM	11.76	0.235	0.136	1.73	0.65			A21.2.2.3	Default	2/3/2006	1:50 PM	375.13	7.503	3.843	1.95	2.22
√	√	A21.2.4	1/23/2006	2:37 PM	22.25	0.445	0.247	1.8	0.71			A21.2.2.4	Default	2/3/2006	1:51 PM	360.63	7.213	3.725	1.94	2.17
√	√	A21.2.5	1/23/2006	2:38 PM	15.34	0.307	0.224	1.37	1.11			A21.2.2.5	Default	2/3/2006	1:52 PM	243.38	4.868	2.519	1.93	2.15
√	√	A21.3.1	1/23/2006	2:39 PM	20.11	0.402	0.215	1.87	0.77			A21.2.3.1	Default	2/3/2006	1:52 PM	240.65	4.813	2.476	1.94	2.22
√	√	A21.3.3	1/23/2006	2:40 PM	20.29	0.406	0.224	1.81	0.8			A21.2.3.2	Default	2/3/2006	1:53 PM	229.58	4.592	2.378	1.93	2.11
√	√	A21.3.4	1/23/2006	2:41 PM	17.22	0.344	0.18	1.91	0.39			A21.2.3.3	Default	2/3/2006	1:54 PM	222.78	4.456	2.291	1.94	2.25
√	√	A21.3.5	1/23/2006	2:41 PM	22.85	0.457	0.248	1.85	0.69			A21.2.3.4	Default	2/3/2006	1:55 PM	367.65	7.353	3.781	1.94	2.24
												A21.2.3.5	Default	2/3/2006	1:57 PM	273.15	5.463	2.815	1.94	2.19

√	√	A22 digested already, Check photo to see if insert?										A22.2.1.1	Default	2/3/2006	1:58 PM	291.19	5.824	3.002	1.94	2.16
												A22.2.1.2	Default	2/3/2006	1:58 PM	197.21	3.944	2.046	1.93	2.17
√	√	A22.1.1	1/23/2006	2:42 PM	37.89	0.758	0.424	1.79	0.49			A22.2.1.3	Default	2/3/2006	1:59 PM	289.55	5.791	2.954	1.96	2.24
√	√	A22.1.2	1/23/2006	2:43 PM	20.07	0.401	0.238	1.69	0.2			A22.2.1.4	Default	2/3/2006	2:00 PM	289.67	5.793	2.988	1.94	2.17
√	√	A22.1.3	1/23/2006	2:44 PM	7.41	0.148	0.156	0.95	0.05			A22.2.1.5	Default	2/3/2006	2:01 PM	291.75	5.835	2.986	1.95	2.27
√	√	A22.1.4	1/23/2006	2:45 PM	9.05	0.181	0.17	1.06	0.14			A22.2.2.1	Default	2/3/2006	2:30 PM	229.96	4.599	2.385	1.93	2.2
√	√	A22.1.5	1/23/2006	2:46 PM	7.52	0.15	0.096	1.56	0.39			A22.2.2.2	Default	2/3/2006	2:30 PM	274.23	5.485	2.827	1.94	2.23
√	√	A22.2.1	1/23/2006	2:46 PM	17.8	0.356	0.23	1.55	0.44			A22.2.2.3	Default	2/3/2006	2:31 PM	175.24	3.505	1.814	1.93	2.2
√	√	A22.2.2	1/23/2006	2:47 PM	18.2	0.364	0.218	1.67	0.25			A22.2.2.4	Default	2/3/2006	2:32 PM	141.45	2.829	1.465	1.93	2.2
√	√	A22.2.3	1/23/2006	2:48 PM	12.75	0.255	0.148	1.72	0.21			A22.2.2.5	Default	2/3/2006	2:33 PM	343.24	6.865	3.534	1.94	2.22
√	√	A22.2.4	1/23/2006	2:49 PM	13.05	0.261	0.15	1.73	0.51			A22.2.3.1	Default	2/3/2006	2:34 PM	169.1	3.382	1.737	1.95	2.24
												A22.2.3.2	Default	2/3/2006	2:02 PM	322.76	6.455	3.321	1.94	2.22
√	√	A23.1.1	1/23/2006	2:50 PM	46.84	0.937	0.574	1.63	0.62			A22.2.3.3	Default	2/3/2006	2:03 PM	175.87	3.517	1.813	1.94	2.21
√	√	A23.1.2	1/23/2006	2:51 PM	17.57	0.351	0.199	1.76	0.53			A22.2.3.4	Default	2/3/2006	2:04 PM	219.58	4.392	2.243	1.96	2.26
√	√	A23.1.3	1/23/2006	2:51 PM	18.03	0.361	0.207	1.75	0.76			A22.2.3.5	Default	2/3/2006	2:04 PM	203.92	4.078	2.113	1.93	2.2
√		A23.1.4	1/23/2006	2:52 PM	-6.23	-0.125	-0.042	2.96	0.42											
√	√	A23.1.5	1/23/2006	2:53 PM	17.05	0.341	0.174	1.96	1.03			A23.2.1.1	Default	2/3/2006	2:35 PM	54.44	1.089	0.565	1.93	2.17
√	√	A23.2.1	1/23/2006	2:54 PM	13.63	0.273	0.182	1.49	0.43			A23.2.1.2	Default	2/3/2006	2:35 PM	42.34	0.847	0.447	1.89	2.01
√	√	A23.2.2	1/20/2006	10:13 PM	153.18	3.064	1.568	1.95	1.98			A23.2.1.3	Default	2/3/2006	2:36 PM	109.08	2.182	1.126	1.94	2.17
√	√	A23.2.3	1/20/2006	10:14 PM	92.58	1.852	0.972	1.91	1.66			A23.2.1.4	Default	2/3/2006	2:37 PM	212.69	4.254	2.162	1.97	2.24
√	√	A23.2.4	1/20/2006	10:14 PM	165.2	3.304	1.716	1.93	1.98			A23.2.1.5	Default	2/3/2006	2:38 PM	128.74	2.575	1.339	1.92	2.2
√	√	A23.2.5	1/20/2006	10:15 PM	164.72	3.294	1.709	1.93	2											
√	√	A23.3.1	1/20/2006	10:16 PM	194.29	3.886	2.021	1.92	1.99											
√	√	A23.3.2	1/20/2006	10:16 PM	258.42	5.168	2.664	1.94	2.06											
√	√	A23.3.3	1/20/2006	10:17 PM	137.16	2.743	1.448	1.89	1.87											
√	√	A23.3.4	1/20/2006	10:17 PM	260.52	5.21	2.686	1.94	2.08											
√	√	A23.3.5	1/20/2006	10:18 PM	166.28	3.326	1.72	1.93	2.02											
√	√	A31 digested already, Check photo to see if insert?																		
												A31.2.1.1	Default	2/3/2006	2:39 PM	40.43	0.809	0.416	1.95	2.13
√		A32.1.3	12/20/2005	12:20 PM	20.42	0.408	0.24	1.7	0.41			A31.2.1.2	Default	2/3/2006	2:40 PM	244.78	4.896	2.507	1.95	2.24
√		A32.1.4	12/20/2005	12:21 PM	10.69	0.214	0.15	1.43	0.28			A31.2.1.3	Default	2/3/2006	2:40 PM	201.23	4.025	2.087	1.93	2.22
√		A32.1.5	12/20/2005	12:22 PM	33.79	0.676	0.392	1.72	0.3			A31.2.1.4	Default	2/3/2006	2:41 PM	139.42	2.788	1.432	1.95	2.22
√		A32.2.1	12/20/2005	12:23 PM	11.18	0.224	0.132	1.7	0.46			A31.2.1.5	Default	2/3/2006	2:42 PM	338.98	6.78	3.494	1.94	2.24
√	√	A32.2.2	12/20/2005	12:24 PM	7.63	0.153	0.103	1.49	0.86			A31.2.2.1	Default	2/3/2006	2:43 PM	117.22	2.344	1.227	1.91	2.24
√		A32.2.3	12/20/2005	12:25 PM	22.38	0.448	0.262	1.71	0.82			A31.2.2.2	Default	2/3/2006	2:43 PM	105.2	2.104	1.054	2	2.23
√	√	A32.2.4	12/20/2005	12:27 PM	14.67	0.293	0.178	1.65	1.81			A31.2.2.3	Default	2/3/2006	2:44 PM	135.16	2.703	1.39	1.94	2.45
√		A32.2.5	12/20/2005	12:27 PM	20.37	0.407	0.216	1.88	0.94			A31.2.2.4	Default	2/3/2006	2:45 PM	170.71	3.414	1.758	1.94	2.23
√		A32.3.1	12/20/2005	12:28 PM	15.12	0.302	0.168	1.8	0.73			A31.2.2.5	Default	2/3/2006	2:46 PM	176.61	3.532	1.812	1.95	2.26
√		A32.3.2	12/20/2005	12:29 PM	31.08	0.622	0.359	1.73	1.26			A31.2.3.1	Default	2/3/2006	2:46 PM	70.88	1.418	0.729	1.94	2.2
√		A32.3.3	12/20/2005	12:30 PM	51.04	1.021	0.552	1.85	1.19			A31.2.3.2	Default	2/3/2006	2:47 PM	133.53	2.671	1.36	1.96	2.25
√		A32.3.5	12/20/2005	12:31 PM	36.82	0.736	0.373	1.98	1.15			A31.2.3.3	Default	2/3/2006	2:48 PM	197.27	3.945	2.057	1.92	2.23
√	√	A32.2.1.1	1/20/2006	8:34 PM	93.45	1.869	0.999	1.87	1.73			A31.2.3.4	Default	2/22/2006	3:23 PM	159.58	3.192	1.581	2.02	3.37
√	√	A32.2.1.2	1/20/2006	8:35 PM	102.07	2.041	1.098	1.86	1.53			A31.2.3.5	Default	2/22/2006	3:25 PM	152.14	3.043	1.612	1.89	1.26
√	√	A32.2.1.3	1/20/2006	8:36 PM	103.21	2.064	1.083	1.91	1.88			A31.2.3.5	Default	2/22/2006	3:26 PM	220.64	4.413	2.19	2.01	2.94
√	√	A32.2.1.4	1/20/2006	8:37 PM	115.46	2.309	1.231	1.88	1.84											
√	√	A32.2.1.5	1/20/2006	8:38 PM	117.77	2.355	1.245	1.89	1.8											
√	√	A32.2.2.1	1/20/2006	8:39 PM	128.95	2.579	1.352	1.91	1.87											
√	√	A32.2.2.2	1/20/2006	8:40 PM	161.94	3.239	1.686	1.92	1.81											
√	√	A32.2.2.3	1/20/2006	8:40 PM	110.46	2.209	1.169	1.89	1.83											
√	√	A32.2.2.4	1/20/2006	8:41 PM	143.26	2.865	1.503	1.91	1.91											
√	√	A32.2.2.5	1/20/2006	8:42 PM	142.71	2.854	1.489	1.92	1.96											
√	√	A32.2.3.1	1/20/2006	8:43 PM	99.13	1.983	1.058	1.87	1.69											
√	√	A32.2.3.2	1/20/2006	8:44 PM	117.16	2.343	1.244	1.88	1.82											
√	√	A32.2.3.3	1/20/2006	8:44 PM	151.13	3.023	1.575	1.92	1.98											
√	√	A32.2.3.4	1/20/2006	8:45 PM	81.82	1.636	0.875	1.87	1.88											
√	√	A32.2.3.5	1/20/2006	8:46 PM	115.69	2.314	1.207	1.92	1.7											
		A33.1.1	12/20/2005	10:55 AM	11.19	0.224	0.106	2.11	0.58			A33.2.1.1	Default	2/22/2006	3:27 PM	19.15	0.383	0.135	2.84	-1.29
		A33.1.2	12/20/2005	10:56 AM	86.4	1.728	0.842	2.05	1.1			A33.2.1.2	Default	2/22/2006	3:37 PM	80.46	1.609	0.776	2.07	2.79
		A33.1.3	12/20/2005	11:03 AM	54.07	1.081	0.538	2.01	1.27			A33.2.1.3	Default	2/22/2006	3:34 PM	77.61	1.552	0.742	2.09	2.69
		A33.1.4	12/20/2005	11:04 AM	101.87	2.037	1.448	1.41	0.37			A33.2.1.4	Default	2/22/2006	3:35 PM	72.54	1.451	0.701	2.07	2.56
		A33.1.5	12/20/2005	11:05 AM	60.41	1.208	0.644	1.88	0.21			A33.2.1.5	Default	2/22/2006	3:36 PM	30.13	0.603	0.251	2.4	4.95
		A33.2.1	12/20/2005	11:06 AM	37.35	0.747	0.367	2.03	1.11			A33.2.2.1	Default	2/22/2006	3:39 PM	42.93	0.859	0.398	2.16	2.64
		A33.2.2	12/20/2005	12:12 PM	33.63	0.673	0.364	1.85	0.47			A33.2.2.2	Default	2/22/2006	3:40 PM	29.46	0.589	0.244	2.42	3.73
		A33.2.3	12/20/2005	12:14 PM	11.17	0.223	0.147	1.52	0.24			A33.2.2.3	Default	2/22/2006	3:40 PM	27.18	0.544	0.227	2.4	5.04
		A33.2.4	12/20/2005	12:15 PM	43.03	0.861	0.496	1.74	0.19			A33.2.2.4	Default	2/22/2006	3:41 PM	23.54	0.471	0.192	2.45	6.11
		A33.3.1	12/20/2005	12:36 PM	70.8	1.416	0.758	1.87	1.19			A33.2.2.5	Default	2/22/2006	3:43 PM	48.96	0.979	0.441	2.22	3.35
		A33.3.2	12/20/2005	12:37 PM	0.99	0.02	0.034	0.58	-1.5			A33.2.3.1	Default	2/22/2006	3:45 PM	52.49	1.05	0.478	2.2	2.96
		A33.3.3	12/20/2005	12:39 PM	20.52	0.41	0.247	1.66	1.01			A33.2.3.2	Default	2/22/2006	3:46 PM	61.98	1.24	0.588	2.11	2.92
		A33.3.4	12/20/2005	12:40 PM	60.23	1.205	0.661	1.82	0.97											
		A33.3.5	12/20/2005	12:41 PM	10.93	0.219	0.156	1.4	0.42											

√		A41.1.1	12/19/2005	6:15 PM	23.26	0.465	0.243	1.92	0.62											
√		A41.1.2	12/19/2005	6:16 PM	-0.58	-0.012	0.003	-3.85	-0.05											
√		A41.1.3	12/19/2005	6:17 PM	38.56	0.771	0.38	2.03	1.56											
√		A41.1.4	12/19/2005	6:19 PM	33.73	0.675	0.341	1.98	1.64											
√	√	A41.1.5	12/19/2005	6:20 PM	57.1	1.142	0.576	1.98	1.34											
√	√	A41.1.6	1/19/2006	2:18 PM	219.91	4.398	2.224	1.98	2.67											
√	√	A41.1.7	1/19/2006	2:19 PM	229.5	4.59	2.345	1.96	2.18											
√	√	A41.1.8	1/19/2006	2:20 PM	101.02	2.02	1.019	1.98	2.11											
√	√	A41.2.1	12/19/2005	6:20 PM	66.16	1.323	0.657	2.01	1.97											
√		A41.2.2	12/19/2005	6:22 PM	39.45	0.789	0.393	2.01	0.57											
√	√	A41.2.3	12/19/2005	6:23 PM	50.43	1.009	0.524	1.92	0.65											
√	√	A41.2.4	12/19/2005	6:23 PM	116.97	2.339	1.202	1.95	1.35											
√	√	A41.2.5	12/19/2005	6:25 PM	52.59	1.052	0.542	1.94	1.03											
√	√	A41.2.6	1/19/2006	2:21 PM	108.34	2.167	1.087	1.99	2.15											
√	√	A41.2.7	1/19/2006	2:22 PM	87.79	1.756	0.906	1.94	1.97											
√	√	A41.2.8	1/19/2006	2:22 PM	224	4.48	2.279	1.97	2.22											
√	√	A41.3.1	12/19/2005	6:25 PM	45.64	0.913	0.487	1.88	1.97											
√	√	A41.3.2	12/19/2005	6:26 PM	23.28	0.466	0.24	1.94	1.11											
√		A41.3.3	12/19/2005	6:27 PM	43.41	0.868	0.513	1.69	0.12											
√		A41.3.4	12/19/2005	6:28 PM	24.49	0.49	0.265	1.85	0.96		A51.3.6	Default	2/22/2006	4:00 PM	175.02	3.5	1.754	2	2.29	
√		A41.3.5	12/19/2005	6:29 PM	6.94	0.139	0.076	1.82	0.53		A51.3.7	Default	2/22/2006	4:00 PM	61.67	1.233	0.607	2.03	2.69	
√	√	A41.3.6	1/19/2006	2:23 PM	151.9	3.038	1.543	1.97	1.96		A51.3.8	Default	2/22/2006	4:01 PM	85.3	1.706	0.842	2.03	2.61	
√	√	A41.3.7	1/19/2006	2:24 PM	126.26	2.525	1.279	1.97	2.16											
√	√	A41.3.8	1/19/2006	2:25 PM	88.8	1.776	0.897	1.98	1.85											
√		A42.1.1	12/20/2005	10:26 AM	11.89	0.238	0.118	2.01	0.9											
√		A42.1.2	12/20/2005	10:27 AM	56.74	1.135	0.569	2	0.62											
√		A42.1.3	12/20/2005	10:28 AM	9.71	0.194	0.108	1.81	0.44											
√		A42.1.3	12/20/2005	10:29 AM	9.51	0.19	0.095	1.99	0.42											
√		A42.1.4	12/20/2005	10:31 AM	40.74	0.815	0.406	2.01	1.05											
√	√	A42.1.5	12/20/2005	10:32 AM	125.78	2.516	1.291	1.95	0.74											
√	√	A42.1.6	1/19/2006	2:26 PM	75.12	1.502	0.766	1.96	2											
√	√	A42.1.7	1/19/2006	2:27 PM	193.56	3.871	1.967	1.97	2.2											
√	√	A42.1.8	1/19/2006	2:28 PM	102.94	2.059	1.041	1.98	2.13											
√		A42.2.1	12/20/2005	10:33 AM	9.55	0.191	0.093	2.06	0.61											
√		A42.2.2	12/20/2005	10:34 AM	83.6	1.672	0.805	2.08	1.19											
√	√	A42.2.3	12/20/2005	10:35 AM	40.74	0.815	0.411	1.98	0.47											
√		A42.2.4	12/20/2005	10:37 AM	24.97	0.499	0.231	2.16	0.62											
√	√	A42.2.6	1/19/2006	2:29 PM	223.35	4.467	2.278	1.96	2.2											
√	√	A42.2.7	1/19/2006	2:29 PM	116.14	2.323	1.173	1.98	2.03											
√	√	A42.2.8	1/19/2006	2:30 PM	250.73	5.015	2.572	1.95	2.18											
√		A42.3.1	12/20/2005	12:18 PM	10.87	0.217	0.14	1.55	0.22											
√	√	A42.3.2	12/20/2005	12:18 PM	85.21	1.704	0.914	1.86	0.89											
√		A42.3.3	12/20/2005	12:19 PM	25.01	0.5	0.282	1.77	0.78											
√	√	A42.3.6	1/19/2006	2:31 PM	336.11	6.722	3.417	1.97	2.2											
√	√	A42.3.7	1/19/2006	2:32 PM	68.61	1.372	0.688	1.99	2.08											
√	√	A42.3.8	1/19/2006	2:32 PM	157.32	3.146	1.597	1.97	2.18											
√	√	A43.1.1	1/23/2006	4:28 PM	30.43	0.609	0.364	1.67	0.8			A43.2.1.1	Default	2/22/2006	3:47 PM	105.8	2.116	1.051	2.01	2.47
√		A43.1.2	1/23/2006	4:34 PM	22.88	0.458	0.293	1.56	0.88			A43.2.1.2	Default	2/22/2006	3:48 PM	196.66	3.933	1.973	1.99	2.43
√	√	A43.1.3	1/23/2006	4:35 PM	52.11	1.042	0.613	1.7	0.54			A43.2.1.3	Default	2/22/2006	3:48 PM	208.58	4.172	2.09	2	2.44
√		A43.1.4	1/23/2006	4:35 PM	7.87	0.157	0.1	1.58	0.66			A43.2.1.4	Default	2/22/2006	3:49 PM	107.28	2.146	1.07	2	2.59
√	√	A43.1.5	1/23/2006	4:36 PM	20.6	0.412	0.256	1.61	0.95			A43.2.1.5	Default	2/22/2006	3:50 PM	195.52	3.91	1.963	1.99	2.43
√	√	A43.2.1	1/23/2006	4:37 PM	16.55	0.331	0.199	1.66	1.14			A43.2.2.1	Default	2/22/2006	3:51 PM	114.44	2.289	1.121	2.04	2.6
√	√	A43.2.2	1/23/2006	4:38 PM	51.77	1.035	0.63	1.64	0.54			A43.2.2.2	Default	2/22/2006	3:52 PM	129.2	2.584	1.276	2.03	2.53
√	√	A43.2.3	1/23/2006	4:39 PM	88.15	1.763	1.125	1.57	0.63			A43.2.2.3	Default	2/22/2006	3:53 PM	195.9	3.918	1.944	2.02	2.48
√		A43.2.4	1/23/2006	4:39 PM	33.32	0.666	0.392	1.7	0.65			A43.2.2.4	Default	2/22/2006	3:54 PM	93.58	1.872	0.916	2.04	2.67
√	√	A43.2.5	1/23/2006	4:40 PM	23.46	0.469	0.256	1.83	1.05			A43.2.2.5	Default	2/22/2006	3:55 PM	178.35	3.567	1.774	2.01	2.42
√	√	A43.3.1	1/23/2006	4:41 PM	25.54	0.511	0.303	1.68	0.73			A43.2.3.1	Default	2/22/2006	3:55 PM	108.49	2.17	1.088	1.99	2.14
√	√	A43.3.2	1/23/2006	4:41 PM	13.31	0.266	0.152	1.75	0.95			A43.2.3.2	Default	2/22/2006	3:56 PM	119.91	2.398	1.218	1.97	2.04
√	√	A43.3.3	1/23/2006	4:42 PM	25.77	0.515	0.293	1.76	0.89			A43.2.3.3	Default	2/22/2006	3:57 PM	121.23	2.425	1.191	2.04	2.55
√	√	A43.3.4	1/23/2006	4:43 PM	24.85	0.497	0.269	1.84	1.08			A43.2.3.4	Default	2/22/2006	3:58 PM	116.27	2.325	1.205	1.93	2.45
√	√	A43.3.5	1/23/2006	4:43 PM	18.44	0.369	0.2	1.84	1.09			A43.2.3.5	Default	2/22/2006	3:59 PM	146.86	2.937	1.463	2.01	2.36

√	√	A51.1.1	1/23/2006	4:45 PM	71.39	1.428	0.76	1.88	1.68											
√	√	A51.1.2	1/23/2006	4:46 PM	97.5	1.95	1.067	1.83	0.77											
√	√	A51.1.3	1/23/2006	4:46 PM	55.5	1.11	0.612	1.81	1.36											
√	√	A51.1.4	1/23/2006	4:47 PM	56.17	1.123	0.609	1.84	1.4											
√	√	A51.1.5	1/23/2006	4:48 PM	58.89	1.178	0.622	1.89	1.51											
√	√	A51.2.1	1/23/2006	4:48 PM	75.2	1.504	0.798	1.89	1.61											
√	√	A51.2.2	1/23/2006	4:49 PM	59.35	1.187	0.644	1.84	1.47											
√	√	A51.2.3	1/23/2006	4:50 PM	63.29	1.266	0.68	1.86	1.73											
√	√	A51.2.4	1/23/2006	4:50 PM	86.09	1.722	0.957	1.8	0.65											
√	√	A51.2.5	1/23/2006	4:51 PM	67.16	1.343	0.724	1.85	1.26											
		A51.2.6	2/2/2006	3:11 PM	106.84	2.137	1.055	2.03	2.12											
		A51.2.7	2/2/2006	3:11 PM	99.05	1.981	0.987	2.01	1.98											
		A51.2.8	2/2/2006	3:13 PM	113.06	2.261	1.137	1.99	2.17											
√	√	A51.3.1	1/23/2006	4:52 PM	64.04	1.281	0.699	1.83	1.05											
√	√	A51.3.2	1/23/2006	4:52 PM	105.85	2.117	1.202	1.76	0.52											
√	√	A51.3.3	1/23/2006	4:53 PM	73.3	1.466	0.827	1.77	1.24											
√	√	A51.3.4	1/23/2006	4:54 PM	12.22	0.244	0.185	1.32	2.24											
√	√	A51.3.5	1/23/2006	4:55 PM	38.43	0.769	0.465	1.65	0.57											
√	√	A51.3.4	1/23/2006	4:55 PM	26.99	0.54	0.32	1.69	0.91											
		A51.3.6	2/22/2006	4:00 PM	175.02	3.5	1.754	2	2.29											
		A51.3.7	2/22/2006	4:00 PM	61.67	1.233	0.607	2.03	2.69											
		A51.3.8	2/22/2006	4:01 PM	85.3	1.706	0.842	2.03	2.61											
√	√	A52.1.1	1/3/2006	2:47 PM	222.87	4.457	2.285	1.95	1.98											
√	√	A52.1.2	1/3/2006	2:48 PM	172.25	3.445	1.754	1.96	2.04											
√	√	A52.1.3	1/3/2006	2:49 PM	87.93	1.759	0.901	1.95	2.66											
√	√	A52.1.4	1/3/2006	2:50 PM	85.67	1.713	0.892	1.92	1.6											
√	√	A52.1.5	1/3/2006	2:51 PM	227.37	4.547	2.314	1.97	2.13											
√	√	A52.2.1	1/3/2006	2:51 PM	216.95	4.339	2.186	1.98	2.17											
√	√	A52.2.2	1/3/2006	2:52 PM	209.65	4.193	2.164	1.94	1.97											
√	√	A52.2.3	1/3/2006	2:53 PM	241.98	4.84	2.461	1.97	2.06											
√	√	A52.2.4	1/3/2006	2:54 PM	187.73	3.755	1.928	1.95	2.05		A51.2.6	Default	2/2/2006	3:11 PM	106.84	2.137	1.055	2.03	2.12
√	√	A52.2.5	1/3/2006	2:55 PM	144.13	2.883	1.479	1.95	1.92		A51.2.7	Default	2/2/2006	3:11 PM	99.05	1.981	0.987	2.01	1.98
√	√	A52.3.1	1/3/2006	2:56 PM	193.03	3.861	1.987	1.94	2.09		A51.2.8	Default	2/2/2006	3:13 PM	113.06	2.261	1.137	1.99	2.17
√	√	A52.3.2	1/3/2006	2:57 PM	145.91	2.918	1.496	1.95	2.04											
√	√	A52.3.3	1/3/2006	2:58 PM	158.94	3.179	1.595	1.99	2.1											
√	√	A52.3.4	1/3/2006	2:58 PM	191.28	3.826	1.955	1.96	2.1											
√	√	A52.3.5	1/3/2006	2:59 PM	184.94	3.699	1.928	1.92	1.9											
√	√	A53.1.1	1/23/2006	4:56 PM	56.64	1.133	0.614	1.85	0.41		A53.1.1	Default	2/2/2006	2:57 PM	36.85	0.737	0.362	2.04	1.79
√	√	A53.1.2	1/23/2006	4:57 PM	36.17	0.723	0.419	1.73	0.61		A53.1.2	Default	2/2/2006	2:58 PM	125.56	2.511	1.263	1.99	2.16
√	√	A53.1.3	1/23/2006	4:58 PM	24.11	0.482	0.287	1.68	1.11		A53.1.3	Default	2/2/2006	2:59 PM	145.57	2.911	1.435	2.03	0.78
√	√	A53.1.4	1/23/2006	4:58 PM	20.57	0.411	0.242	1.7	0.78		A53.1.4	Default	2/2/2006	3:00 PM	96.97	1.939	0.956	2.03	2.14
√	√	A53.1.5	1/23/2006	4:59 PM	25.85	0.517	0.286	1.81	0.95		A53.1.5	Default	2/2/2006	3:01 PM	96.33	1.927	0.962	2	2.15
√	√	A53.2.1	1/23/2006	4:59 PM	25.16	0.503	0.306	1.64	1.06		A53.2.1	Default	2/2/2006	3:02 PM	131.8	2.636	1.329	1.98	2.15
√	√	A53.2.2	1/23/2006	5:00 PM	24.53	0.491	0.278	1.76	0.69		A53.2.2	Default	2/2/2006	3:03 PM	103.56	2.071	1.037	2	2.14
√	√	A53.2.3	1/23/2006	5:01 PM	24.5	0.49	0.276	1.78	0.71		A53.2.3	Default	2/2/2006	3:03 PM	92.06	1.841	0.928	1.98	2.04
√		A53.2.4	1/23/2006	5:01 PM	8.26	0.165	0.108	1.53	0.58		A53.2.4	Default	2/2/2006	3:04 PM	48.02	0.96	0.474	2.03	2.01
√	√	A53.2.5	1/23/2006	5:02 PM	18.88	0.378	0.218	1.73	1.05		A53.2.5	Default	2/2/2006	3:05 PM	50.39	1.008	0.497	2.03	2.06
√	√	A53.3.1	1/23/2006	5:03 PM	13.58	0.272	0.184	1.48	0.57		A53.3.1	Default	2/2/2006	3:06 PM	64.11	1.282	0.644	1.99	2.02
√	√	A53.3.2	1/23/2006	5:03 PM	27.01	0.54	0.318	1.7	0.63		A53.3.2	Default	2/2/2006	3:07 PM	104.71	2.094	1.038	2.02	2.17
√	√	A53.3.3	1/23/2006	5:04 PM	17.54	0.351	0.21	1.67	0.94		A53.3.3	Default	2/2/2006	3:08 PM	79.52	1.59	0.783	2.03	2.12
√	√	A53.3.4	1/23/2006	5:05 PM	38.57	0.771	0.46	1.68	0.82		A53.3.4	Default	2/2/2006	3:09 PM	91.41	1.828	0.914	2	2.13
√	√	A53.3.5	1/23/2006	5:05 PM	19.3	0.386	0.225	1.72	0.84		A53.3.5	Default	2/2/2006	3:10 PM	64.12	1.282	0.657	1.95	2.07
√	√	A61.1.1	1/3/2006	3:00 PM	140.12	2.802	1.429	1.96	2.12											
√	√	A61.1.2	1/3/2006	3:01 PM	159.2	3.184	1.64	1.94	2.06											
√	√	A61.1.3	1/3/2006	3:02 PM	191.03	3.821	2.099	1.82	1.27											
√	√	A61.1.4	1/3/2006	3:04 PM	206.11	4.122	2.095	1.97	2.13											
√	√	A61.1.5	1/3/2006	3:05 PM	162.8	3.256	1.656	1.97	2.24											
√	√	A61.2.1	1/3/2006	3:05 PM	203.72	4.074	2.082	1.96	1.98											
√	√	A61.2.2	1/3/2006	3:06 PM	162.73	3.255	1.655	1.97	2.13											
√	√	A61.2.3	1/3/2006	3:07 PM	239.16	4.783	2.444	1.96	2.08											
√	√	A61.2.5	1/3/2006	3:08 PM	173.86	3.477	1.784	1.95	2.1											
√	√	A61.3.1	1/3/2006	3:09 PM	240.04	4.801	2.473	1.94	2.09											
√	√	A61.3.2	1/3/2006	3:10 PM	217.81	4.356	2.256	1.93	1.94											
√	√	A61.3.3	1/3/2006	3:10 PM	240.96	4.819	2.45	1.97	2.07											
√	√	A61.3.4	1/3/2006	3:12 PM	166.26	3.325	1.709	1.95	2.03											
√	√	A61.3.5	1/3/2006	3:13 PM	227.81	4.556	2.359	1.93	1.86											

√	√	A62.1.1	1/20/2006	8:47 PM	164.71	3.294	1.699	1.94	1.99
√	√	A62.1.2	1/20/2006	8:48 PM	135.78	2.716	1.433	1.9	1.94
√	√	A62.1.3	1/20/2006	8:49 PM	134.41	2.688	1.405	1.91	1.64
√	√	A62.1.4	1/20/2006	8:50 PM	152.98	3.06	1.599	1.91	1.99
√	√	A62.1.5	1/20/2006	8:50 PM	152.16	3.043	1.62	1.88	1.88
√	√	A62.2.1	1/20/2006	8:51 PM	117.5	2.35	1.246	1.89	1.75
√	√	A62.2.2	1/20/2006	8:52 PM	115.39	2.308	1.234	1.87	1.69
√	√	A62.2.3	1/20/2006	8:52 PM	183.85	3.677	1.914	1.92	1.74
√	√	A62.2.4	1/20/2006	8:53 PM	102.03	2.041	1.062	1.92	1.82
√	√	A62.2.5	1/20/2006	8:54 PM	76.29	1.526	0.803	1.9	1.73
√	√	A62.3.1	1/20/2006	8:54 PM	89.27	1.785	0.94	1.9	1.81
√	√	A62.3.2	1/20/2006	8:55 PM	67.97	1.359	0.683	1.99	1.78
√	√	A62.3.3	1/20/2006	8:56 PM	138.32	2.766	1.457	1.9	1.88
√	√	A62.3.4	1/20/2006	8:57 PM	91.28	1.826	0.939	1.94	1.53
√	√	A62.3.5	1/20/2006	8:57 PM	108.74	2.175	1.155	1.88	1.89
√	√	A63.1.1	1/20/2006	8:58 PM	104.21	2.084	1.1	1.89	1.51
√	√	A63.1.2	1/20/2006	8:59 PM	99.23	1.985	1.039	1.91	1.49
√	√	A63.1.3	1/20/2006	8:59 PM	170.32	3.406	1.773	1.92	1.77
√	√	A63.1.4	1/20/2006	9:00 PM	103.85	2.077	1.089	1.91	1.81
√	√	A63.1.5	1/20/2006	9:01 PM	106.03	2.121	1.125	1.88	1.82
√	√	A63.2.1	1/20/2006	9:02 PM	74.77	1.495	0.813	1.84	1.76
√	√	A63.2.2	1/20/2006	9:02 PM	173.04	3.461	1.803	1.92	1.96
√	√	A63.2.3	1/20/2006	9:03 PM	135.15	2.703	1.432	1.89	1.81
√	√	A63.2.4	1/20/2006	9:04 PM	111.97	2.239	1.184	1.89	1.78
√	√	A63.2.5	1/20/2006	9:05 PM	76.76	1.535	0.826	1.86	1.76
√	√	A63.3.1	1/20/2006	9:05 PM	122.18	2.444	1.3	1.88	1.7
√	√	A63.3.2	1/20/2006	9:50 PM	345.09	6.902	3.612	1.91	2.03
√	√	A63.3.2	1/20/2006	9:53 PM	320.03	6.401	3.304	1.94	2.06
√	√	A63.3.3	1/20/2006	9:54 PM	184.94	3.699	1.923	1.92	2.02
√	√	A63.3.4	1/20/2006	9:54 PM	144.12	2.882	1.508	1.91	1.95
√	√	A63.3.5	1/20/2006	9:55 PM	259.24	5.185	2.678	1.94	2.06
√	√	A71.1.2	12/8/2005	11:17 AM	97.7	4.066	2.131	1.91	2.02
√	√	TE buffer	12/8/2005	11:21 AM	-0.93	-0.019	0.005	-3.57	-28.37
√	√	A71.1.3	12/8/2005	11:24 AM	179.51	3.59	1.891	1.9	2.01
√	√	A71.1.4	12/8/2005	11:26 AM	224.78	4.496	2.333	1.93	2.04
√	√	A71.1.5	12/8/2005	11:28 AM	236.6	4.732	2.443	1.94	2.21
√	√	A71.1.1	12/8/2005	11:29 AM	150.03	3.001	1.585	1.89	2.13
√	√	A71.1.5	12/8/2005	11:34 AM	238.92	4.778	2.474	1.93	2.22
√	√	A71.2.1	12/8/2005	11:35 AM	161.69	3.234	1.653	1.96	2.21
√	√	A71.2.2	12/8/2005	11:37 AM	175.09	3.502	1.775	1.97	2.26
√	√	A71.2.4	12/8/2005	11:39 AM	197.98	3.96	2.016	1.96	2.25
√	√	A71.2.3	12/8/2005	11:41 AM	142.52	2.85	1.458	1.95	2.22
√	√	A71.2.5	12/8/2005	11:42 AM	166.39	3.328	1.695	1.96	2.23
√	√	A71.3.1	12/8/2005	11:43 AM	113.69	2.274	1.168	1.95	2.1
√	√	A71.3.2	12/8/2005	11:45 AM	112.31	2.246	1.167	1.92	2.2
√	√	A71.3.3	12/8/2005	11:46 AM	152.81	3.056	1.581	1.93	2.18
√	√	A71.3.4	12/8/2005	11:47 AM	122.46	2.449	1.231	1.99	2.32
√	√	A71.3.5	12/8/2005	11:48 AM	156.85	3.137	1.608	1.95	2.21
√		NC A71	12/8/2005	11:49 AM	141.31	2.826	1.446	1.95	2.26
√	√	A72.1.1	12/8/2005	11:51 AM	124.9	2.498	1.331	1.88	1.84
√	√	A72.1.2	12/8/2005	11:52 AM	137.44	2.749	1.393	1.97	2.91
√	√	A72.1.3	12/8/2005	11:53 AM	159.79	3.196	1.641	1.95	2.27
√	√	A72.1.4	12/8/2005	11:54 AM	171.72	3.434	1.762	1.95	2.26
√	√	A72.1.5	12/8/2005	11:55 AM	164.89	3.298	1.701	1.94	2.13
√	√	A72.2.1	12/8/2005	11:57 AM	157.88	3.158	1.625	1.94	2.21
√	√	A72.2.2	12/8/2005	11:58 AM	273.23	5.465	2.81	1.94	2.25
√	√	A72.2.3	12/8/2005	11:59 AM	223.34	4.467	2.313	1.93	2.24
√	√	A72.2.4	12/8/2005	12:00 PM	123.89	2.478	1.264	1.96	3.19
√	√	A72.2.5	12/8/2005	12:01 PM	249.01	4.98	2.576	1.93	2.25
√	√	A72.3.1	12/8/2005	12:02 PM	106.22	2.124	1.096	1.94	2.46
√	√	A72.3.2	12/8/2005	12:15 PM	159.1	3.182	1.616	1.97	2.64
√	√	A72.3.3	12/8/2005	12:16 PM	108.32	2.166	1.094	1.98	3.3
√	√	A72.3.4	12/8/2005	12:17 PM	133.9	2.678	1.365	1.96	2.34
√	√	A72.3.5	12/8/2005	12:18 PM	175.48	3.51	1.793	1.96	2.3
√		NC A72	12/8/2005	12:19 PM	69.46	1.389	0.699	1.99	2.61

√	√	A73.1.1	12/8/2005	12:20 PM	50.44	1.009	0.472	2.14	2.05
√	√	TE Buffer	12/8/2005	12:23 PM	-1	-0.02	-0.033	0.6	0.92
√	√	NC A72	12/8/2005	12:26 PM	71.18	1.424	0.73	1.95	2.5
√	√	A73.1.2	12/8/2005	12:28 PM	37.24	0.745	0.382	1.95	2.13
√	√	A73.1.3	12/8/2005	12:29 PM	32.87	0.657	0.358	1.84	2.38
√	√	A73.1.4	12/8/2005	12:30 PM	73.87	1.477	0.767	1.93	2.25
√	√	A73.1.5	12/8/2005	12:31 PM	21.48	0.43	0.248	1.73	2.09
√	√	A72.3.5	12/8/2005	12:36 PM	180.81	3.616	1.863	1.94	2.3
√	√	A73.2.1	12/8/2005	12:38 PM	7.66	0.153	0.072	2.13	1.91
√	√	A73.2.2	12/8/2005	12:40 PM	53.11	1.062	0.553	1.92	2.34
√	√	A73.2.3	12/8/2005	12:41 PM	128.07	2.561	1.317	1.94	2.31
√	√	A73.2.4	12/8/2005	12:42 PM	96.02	1.92	0.999	1.92	2.19
√	√	A73.2.5	12/8/2005	12:43 PM	31.15	0.623	0.329	1.89	2.46
√	√	A73.3.1	12/8/2005	12:50 PM	59.56	1.191	0.609	1.96	2
√	√	A73.3.2	12/8/2005	12:51 PM	70.4	2.479	1.298	1.91	2.33
√	√	A73.3.3	12/8/2005	12:52 PM	74.11	1.482	0.773	1.92	2.22
√	√	A73.3.4	12/8/2005	12:53 PM	102.76	2.055	1.063	1.93	2.23
√	√	A73.3.5	12/8/2005	12:54 PM	68.25	1.365	0.715	1.91	2.29
√		NC A73	12/8/2005	12:55 PM	74.01	1.48	0.764	1.94	2.33
√	√	A81.1.1	12/8/2005	12:56 PM	97.13	1.943	1.012	1.92	2.04
√	√	A81.1.2	12/8/2005	12:57 PM	93.54	1.871	0.946	1.98	3.17
√	√	A81.1.3	12/8/2005	12:58 PM	64.52	1.29	0.674	1.91	3.74
√	√	A81.1.4	12/8/2005	12:59 PM	102.05	2.041	1.034	1.97	3.28
√	√	A81.1.5	12/8/2005	1:00 PM	137.93	2.759	1.417	1.95	2.62
√	√	A81.2.1	12/8/2005	1:01 PM	107.87	2.157	1.108	1.95	2.49
√	√	A81.2.2	12/8/2005	1:02 PM	149.71	2.994	1.546	1.94	2.17
√	√	A81.2.3	12/8/2005	1:03 PM	170.72	3.414	1.76	1.94	2.14
√	√	A81.2.4	12/8/2005	1:04 PM	156.62	3.132	1.593	1.97	2.85
√	√	A81.2.5	12/8/2005	1:05 PM	106.45	2.129	1.096	1.94	2.27
√	√	A81.3.1	12/8/2005	1:06 PM	139.94	2.799	1.474	1.9	2.09
√	√	A81.3.2	12/8/2005	1:07 PM	127.73	2.555	1.32	1.94	2.13
√	√	A81.3.3	12/8/2005	1:09 PM	145.01	2.9	1.517	1.91	2.14
√	√	A81.3.4	12/8/2005	1:10 PM	183.21	3.664	1.895	1.93	2.18
√	√	A81.3.5	12/8/2005	1:12 PM	202.39	4.048	2.06	1.96	2.8
√		NC A81	12/8/2005	1:13 PM	91.41	1.828	0.974	1.88	2.29
√	√	A82.1.1	12/8/2005	1:15 PM	146.93	2.939	1.541	1.91	1.94
√	√	A82.1.2	12/8/2005	1:24 PM	142.91	2.858	1.444	1.98	2.5
√	√	A82.1.3	12/8/2005	1:27 PM	137.58	2.752	1.401	1.96	2.86
√	√	A82.1.4	12/8/2005	1:28 PM	126.99	2.54	1.286	1.98	2.88
√	√	A82.1.5	12/8/2005	1:29 PM	97.03	1.941	0.993	1.95	2.58
√	√	A82.2.1	12/8/2005	1:30 PM	104.02	2.08	1.187	1.75	1.1
√	√	A82.2.2	12/8/2005	1:31 PM	106.3	2.126	1.071	1.98	3.43
√	√	A82.2.3	12/8/2005	1:32 PM	127.93	2.559	1.299	1.97	2.88
√	√	A82.2.4	12/8/2005	1:33 PM	217.02	4.34	2.183	1.99	2.71
√	√	A82.2.5	12/13/2005	11:09 AM	203.81	4.076	2.138	1.91	1.84
√	√	A82.3.1	12/13/2005	11:10 AM	185.84	3.717	1.918	1.94	2.21
√	√	A82.3.2	12/13/2005	11:11 AM	217.34	4.347	2.263	1.92	2.05
√	√	A82.3.3	12/13/2005	11:12 AM	177.43	3.549	1.832	1.94	1.97
√	√	A82.3.4	12/13/2005	11:13 AM	214.79	4.296	2.245	1.91	2.07
√	√	A82.3.5	12/13/2005	11:14 AM	99.3	1.986	1.057	1.88	2.05
√	√	A83.1.1	12/13/2005	11:15 AM	303.18	6.064	3.107	1.95	2.17
√	√	A83.1.2	12/13/2005	11:18 AM	133.38	2.668	1.441	1.85	2.53
√	√	A83.1.3	12/13/2005	11:19 AM	209.72	4.194	2.141	1.96	2.29
√	√	A83.1.4	12/13/2005	11:20 AM	166.58	3.332	1.713	1.94	2.47
√	√	A83.1.5	12/13/2005	11:21 AM	186.25	3.725	1.932	1.93	2.22
√	√	A83.2.1	12/13/2005	2:20 PM	10.62	0.212	0.101	2.1	0.62
√	√	A83.2.2	12/13/2005	2:21 PM	27.55	0.551	0.283	1.95	1.49
√	√	A83.2.3	12/13/2005	2:24 PM	14.29	0.286	0.146	1.96	0.93
√	√	A83.2.4	12/13/2005	2:26 PM	51.67	1.033	0.545	1.9	1.57
√	√	A83.2.5	12/13/2005	2:27 PM	166.08	3.322	1.571	2.11	1.89
√	√	A83.3.1	12/13/2005	2:29 PM	14.73	0.295	0.159	1.85	0.93
√	√	A83.3.2	12/13/2005	2:32 PM	24.59	0.492	0.243	2.02	1.06
√	√	A83.3.3	12/13/2005	2:33 PM	91.1	1.822	0.871	2.09	1.35
√	√	A83.3.4	12/13/2005	2:38 PM	24.48	0.49	0.243	2.01	1.48
√	√	A83.3.5	12/13/2005	2:36 PM	8.17	0.163	0.102	1.6	1.4
√		A83 NC	12/13/2005	11:44 AM	0.98	0.02	0.025	0.79	-0.29

√	√	A91.1.1	12/13/2005	11:46 AM	126.2	2.524	1.314	1.92	2.1
√	√	A91.1.2	12/13/2005	11:48 AM	258.18	5.164	2.689	1.92	1.97
√	√	A91.1.3	12/13/2005	11:49 AM	215.12	4.302	2.187	1.97	2.53
√	√	A91.1.4	12/13/2005	11:51 AM	228.1	4.562	2.342	1.95	2.15
√	√	A91.1.5	12/13/2005	11:53 AM	193.82	3.876	2.027	1.91	1.82
√	√	A91.2.1	12/13/2005	11:58 AM	227.34	4.547	2.328	1.95	2.1
√	√	A91.2.2	12/13/2005	11:59 AM	387.06	7.741	3.975	1.95	2.07
√	√	A91.2.3	12/13/2005	12:00 PM	1142.1	22.841	11.642	1.96	2.24
√	√	A91.2.3	12/13/2005	12:01 PM	1071.8	21.436	10.945	1.96	2.24
√	√	A91.2.3	12/13/2005	12:02 PM	389.11	7.782	4.043	1.93	2.15
√	√	A91.2.4	12/13/2005	12:03 PM	170.7	3.414	1.798	1.9	1.9
√	√	A91.2.3	12/13/2005	12:05 PM	552.99	11.06	5.771	1.92	2.02
√	√	A91.2.5	12/13/2005	12:06 PM	407.89	8.158	4.193	1.95	2.12
√	√	A91.3.1	12/13/2005	12:08 PM	291.9	5.838	2.995	1.95	2.02
√	√	A91.3.2	12/13/2005	12:09 PM	823.43	16.469	8.407	1.96	2.22
√	√	A91.3.3	12/13/2005	12:11 PM	247.86	4.957	2.537	1.95	1.99
√	√	A91.3.5	12/13/2005	2:40 PM	415.04	8.301	4.267	1.95	2.29
√	√	A92.1.1	12/13/2005	2:41 PM	789.17	15.783	8.092	1.95	2.21
√	√	A92.1.2	12/13/2005	2:42 PM	419.25	8.385	4.31	1.95	2.29
√	√	A92.1.3	12/13/2005	2:44 PM	374.12	7.482	3.87	1.93	2.11
√	√	A92.1.5	12/13/2005	2:45 PM	538.09	10.762	5.626	1.91	2.09
√	√	A92.2.1	12/13/2005	2:47 PM	240.51	4.81	2.483	1.94	2.36
√	√	A92.2.2	12/13/2005	2:47 PM	390.68	7.814	4.035	1.94	2.04
√	√	A92.2.3	12/13/2005	2:49 PM	381.17	7.623	3.956	1.93	2.16
√	√	A92.2.4	12/13/2005	2:50 PM	345.96	6.919	3.546	1.95	2.31
√	√	A92.2.5	12/13/2005	2:51 PM	679.46	13.589	6.837	1.99	2.21
√	√	A92.3.1	12/13/2005	2:52 PM	690.75	13.815	6.94	1.99	2.22
√	√	A92.3.2	12/13/2005	2:53 PM	437.97	8.759	4.558	1.92	2.12
√	√	A92.3.3	12/13/2005	2:54 PM	314.44	6.289	3.243	1.94	2.14
√	√	A92.3.4	12/13/2005	2:55 PM	499.72	9.994	5.205	1.92	2.07
√	√	A92.3.5	12/13/2005	2:56 PM	447.02	8.94	4.639	1.93	2.11
√	√	A93.1.1	12/13/2005	2:57 PM	356.78	7.136	3.68	1.94	2.1
√	√	A93.1.2	12/13/2005	2:59 PM	685.26	13.705	6.972	1.97	2.16
√	√	A93.1.3	12/15/2005	1:34 PM	432.22	8.644	4.443	1.95	2.12
√	√	A93.1.4	12/15/2005	1:35 PM	635.99	12.72	6.536	1.95	2.19
√	√	A93.1.5	12/15/2005	1:33 PM	421.58	8.432	4.402	1.92	1.89
√	√	A93.2.1.5	12/30/2005	10:57 AM	676.2	13.524	6.995	1.93	2.21
√	√	A93.2.1	12/15/2005	1:36 PM	484.79	9.696	5.105	1.9	1.95
√	√	A93.2.2	12/15/2005	1:38 PM	536.32	10.726	5.583	1.92	2.16
√	√	A93.2.3	12/15/2005	1:39 PM	425.39	8.508	4.481	1.9	1.8
√	√	A93.2.2.3	12/30/2005	10:58 AM	423.32	8.466	4.398	1.93	2.14
√	√	A93.2.4	12/15/2005	1:40 PM	489.69	9.794	5.121	1.91	2.09
√	√	A93.2.5	12/15/2005	1:41 PM	570.64	11.413	5.977	1.91	2.13
√	√	A93.3.1	12/15/2005	1:42 PM	534.4	10.688	5.573	1.92	2.15
√	√	A93.3.2	12/15/2005	1:43 PM	257.63	5.153	2.685	1.92	1.97
√	√	A93.3.3	12/15/2005	1:44 PM	535.62	10.712	5.551	1.93	2.18
√	√	A93.3.4	12/15/2005	2:02 PM	525.08	10.502	5.533	1.9	2.1
√	√	A93.3.5	12/15/2005	2:04 PM	478.89	9.578	4.96	1.93	2.17
√	√	A10-1.1.1	12/15/2005	1:22 PM	468.31	9.366	4.899	1.91	1.99
√	√	A10-1.1.2	12/15/2005	1:23 PM	1227.6	24.551	12.614	1.95	2.25
√	√	A10-1.1.3	12/15/2005	1:24 PM	553.76	11.075	5.769	1.92	2.18
		A10-1.1.5	12/15/2005	1:25 PM	398.15	7.963	4.16	1.91	1.9
√	√	A10-1.2.1	12/15/2005	1:27 PM	502.54	10.051	5.252	1.91	2.14
√	√	A10-1.2.2	12/15/2005	1:28 PM	298.73	5.975	3.076	1.94	2.17
√	√	A10-1.2.3	12/15/2005	1:29 PM	600.54	12.011	6.302	1.91	2.12
√	√	A10-1.2.4	12/15/2005	1:30 PM	697.3	13.946	7.125	1.96	2.22
√	√	A10-1.2.5	12/15/2005	1:31 PM	482.15	9.643	5.072	1.9	1.84
√	√	A10-1.2.6	1/19/2006	2:33 PM	401.35	8.027	4.114	1.95	2.22
√	√	A10-1.2.7	1/19/2006	2:34 PM	258.24	5.165	2.644	1.95	2.2
√		A10-1.3.1	12/30/2005	11:16 AM	13.32	0.266	0.126	2.12	1
√	√	A10-1.3.2	12/30/2005	11:17 AM	464.77	9.295	4.833	1.92	2.16
√	√	A10-1.3.3	12/30/2005	11:18 AM	529.85	10.597	5.517	1.92	2.14
√	√	A10-1.3.4	12/30/2005	11:18 AM	65.33	1.307	0.672	1.95	1.8
√	√	A10-1.3.5	12/30/2005	11:19 AM	395.61	7.912	4.078	1.94	2.13

√	√	A10-2.1.1	12/30/2005	11:00 AM	177.95	3.559	1.825	1.95	2.52
√	√	A10-2.1.2	12/30/2005	11:04 AM	260.19	5.204	2.68	1.94	2.08
√	√	A10-2.1.3	12/30/2005	11:05 AM	242.51	4.85	2.495	1.94	2.13
√	√	A10-2.1.4	12/30/2005	11:06 AM	586.94	11.739	6.164	1.9	2.03
√	√	A10-2.1.5	12/30/2005	11:07 AM	404.96	8.099	4.181	1.94	2.14
√	√	A10-2.2.1	12/30/2005	11:08 AM	296.45	5.929	3.05	1.94	2.11
√	√	A10-2.2.2	12/30/2005	11:08 AM	392.19	7.844	4.042	1.94	2.16
√	√	A10-2.2.3	12/30/2005	11:09 AM	238.55	4.771	2.463	1.94	2.11
√	√	A10-2.2.4	12/30/2005	11:10 AM	238.69	4.774	2.439	1.96	2.11
√	√	A10-2.2.5	12/30/2005	11:11 AM	252.78	5.056	2.608	1.94	2.13
√	√	A10-2.2.6	1/19/2006	2:35 PM	403.3	8.066	4.204	1.92	2.21
√	√	A10-2.2.7	1/19/2006	2:36 PM	425.13	8.503	4.363	1.95	2.23
√	√	A10-2.3.1	12/30/2005	11:12 AM	100.79	2.016	1.065	1.89	1.92
√	√	A10-2.3.2	12/30/2005	11:12 AM	345.78	6.916	3.566	1.94	2.03
√	√	A10-2.3.3	12/30/2005	11:13 AM	325.25	6.505	3.383	1.92	2.04
√	√	A10-2.3.4	12/30/2005	11:14 AM	293.36	5.867	3.028	1.94	2.14
√	√	A10-2.3.5	12/30/2005	11:15 AM	418.17	8.363	4.338	1.93	2.17
√	√	A10-3.1.1	12/30/2005	11:20 AM	283.85	5.677	2.932	1.94	2.07
√	√	A10-3.1.2	12/30/2005	11:21 AM	450.48	9.01	4.693	1.92	2.11
√	√	A10-3.1.3	12/30/2005	11:21 AM	524.22	10.484	5.46	1.92	2.11
√	√	A10-3.1.4	12/30/2005	11:22 AM	441.63	8.833	4.562	1.94	2.13
√	√	A10-3.1.5	12/30/2005	11:25 AM	353.6	7.072	3.644	1.94	2.13
√	√	A10-3.2.1	12/30/2005	11:26 AM	403.32	8.066	4.215	1.91	2.14
√	√	A10-3.2.2	12/30/2005	11:26 AM	183.13	3.663	1.902	1.93	2.08
√	√	A10-3.2.3	12/30/2005	11:27 AM	282.18	5.644	2.896	1.95	2.08
√	√	A10-3.2.4	12/30/2005	11:28 AM	306.5	6.13	3.186	1.92	2.06
√	√	A10-3.2.5	12/30/2005	11:28 AM	479.7	9.594	4.977	1.93	2.2
√	√	A10-3.3.1	12/30/2005	11:29 AM	239.42	4.788	2.454	1.95	2.1
√	√	A10-3.3.2	12/30/2005	11:30 AM	428.54	8.571	4.393	1.95	2.17
√	√	A10-3.3.3	12/30/2005	11:30 AM	255.46	5.109	2.624	1.95	2.1
√	√	A10-3.3.4	12/30/2005	11:31 AM	329.55	6.591	3.422	1.93	2.09
√	√	A10-3.3.5	12/30/2005	11:32 AM	105.46	2.109	1.094	1.93	2.06
		A10-3.3.6	1/19/2006	2:37 PM	330.57	6.611	3.382	1.95	2.22
		A10-3.3.7	1/19/2006	2:38 PM	249.92	4.998	2.562	1.95	2.14
√	√	A11-1.1.1	1/3/2006	3:14 PM	188.42	3.768	1.907	1.98	2.25
√	√	A11-1.1.2	1/3/2006	3:14 PM	216.31	4.326	2.186	1.98	2.22
√	√	A11-1.1.3	1/3/2006	3:15 PM	206.78	4.136	2.109	1.96	2.16
√	√	A11-1.1.4	1/3/2006	3:16 PM	188.84	3.777	1.91	1.98	2.14
√	√	A11-1.1.5	1/3/2006	3:17 PM	199.58	3.992	2.027	1.97	2.18
		A11-1.1.6	1/19/2006	2:07 PM	305.99	6.12	3.163	1.93	2.13
		A11-1.1.7	1/19/2006	2:06 PM	429.53	8.591	4.425	1.94	2.19
√	√	A11-1.2.1	1/3/2006	3:18 PM	222.14	4.443	2.298	1.93	2.11
√	√	A11-1.2.2	1/3/2006	3:19 PM	180.64	3.613	1.847	1.96	2.18
√	√	A11-1.2.3	1/3/2006	3:20 PM	156.83	3.137	1.602	1.96	2.2
√	√	A11-1.2.4	1/3/2006	3:20 PM	201.09	4.022	2.065	1.95	1.84
√	√	A11-1.2.5	1/3/2006	3:21 PM	245.15	4.903	2.507	1.96	2.12
√	√	A11-1.3.1	1/3/2006	3:22 PM	215.46	4.309	2.197	1.96	1.91
√	√	A11-1.3.2	1/3/2006	3:23 PM	156.78	3.136	1.633	1.92	1.79
√	√	A11-1.3.3	1/3/2006	3:24 PM	150.06	3.001	1.545	1.94	2.01
√	√	A11-1.3.4	1/3/2006	3:25 PM	183.3	3.666	1.878	1.95	2.07
√	√	A11-1.3.5	1/3/2006	3:25 PM	208.4	4.168	2.115	1.97	2.18

√	√	A11-2.1.1	1/20/2006	9:56 PM	260.86	5.217	2.711	1.92	2.07
√	√	A11-2.1.2	1/20/2006	9:56 PM	178.85	3.577	1.853	1.93	1.94
√	√	A11-2.1.3	1/20/2006	9:57 PM	198.34	3.967	2.062	1.92	2.01
√	√	A11-2.1.4	1/20/2006	9:58 PM	192.75	3.855	1.991	1.94	2.02
√	√	A11-2.1.5	1/20/2006	9:58 PM	213.45	4.269	2.203	1.94	2.01
√	√	A11-2.2.1	1/20/2006	9:59 PM	179.37	3.587	1.842	1.95	2.01
√	√	A11-2.2.2	1/20/2006	10:00 PM	302.21	6.044	3.106	1.95	2.08
√	√	A11-2.2.3	1/20/2006	10:01 PM	206.54	4.131	2.132	1.94	2
√	√	A11-2.2.4	1/20/2006	10:02 PM	165.05	3.301	1.71	1.93	1.96
√	√	A11-2.2.5	1/20/2006	10:02 PM	104.33	2.087	1.094	1.91	1.82
√	√	A11-2.3.1	1/20/2006	10:03 PM	150.7	3.014	1.573	1.92	1.98
√	√	A11-2.3.2	1/20/2006	10:04 PM	244.07	4.881	2.509	1.95	2.08
√	√	A11-2.3.3	1/20/2006	10:04 PM	194.52	3.89	2.01	1.94	2.03
√	√	A11-2.3.4	1/20/2006	10:05 PM	171.06	3.421	1.782	1.92	1.92
√	√	A11-2.3.5	1/20/2006	10:05 PM	122.7	2.454	1.275	1.92	1.85
√	√	A11-3.1.1	1/9/2006	5:27 PM	130.18	2.604	1.4	1.86	1.37
√	√	A11-3.1.2	1/9/2006	5:28 PM	73.85	1.477	0.789	1.87	1.68
√	√	A11-3.1.3	1/9/2006	5:29 PM	87.68	1.754	0.969	1.81	1.16
√	√	A11-3.1.4	1/9/2006	5:30 PM	95.68	1.914	1.067	1.79	2.09
√	√	A11-3.1.5	1/9/2006	5:31 PM	77.41	1.548	0.784	1.98	2.25
√	√	A11-3.2.1	1/9/2006	5:31 PM	111.88	2.238	1.23	1.82	1.23
√	√	A11-3.2.2	1/9/2006	5:32 PM	142.16	2.843	1.563	1.82	1.3
√	√	A11-3.2.3	1/9/2006	5:33 PM	141.64	2.833	1.558	1.82	1.4
√	√	A11-3.2.4	1/9/2006	5:34 PM	102.34	2.047	1.107	1.85	1.44
√	√	A11-3.2.5	1/9/2006	5:35 PM	128.5	2.57	1.347	1.91	1.76
√	√	A11-3.3.1	1/9/2006	5:36 PM	84.11	1.682	0.865	1.94	2.06
√	√	A11-3.3.2	1/9/2006	5:38 PM	103.54	2.071	1.141	1.82	1.16
√	√	A11-3.3.3	1/9/2006	5:38 PM	149.84	2.997	1.629	1.84	1.21
√	√	A11-3.3.4	1/9/2006	5:39 PM	104.05	2.081	1.139	1.83	1.22
√	√	A11-3.3.5	1/9/2006	5:40 PM	111.54	2.231	1.214	1.84	1.46
		A12-1.1.1	1/19/2006	1:26 PM	347.21	6.944	3.559	1.95	2.19
		A12-1.1.2	1/19/2006	1:27 PM	285.19	5.704	2.916	1.96	2.15
		A12-1.1.3	1/19/2006	1:28 PM	407.3	8.146	4.2	1.94	2.13
		A12-1.1.4	1/19/2006	1:29 PM	262.11	5.242	2.681	1.96	2.19
		A12-1.1.5	1/19/2006	1:30 PM	254.92	5.098	2.611	1.95	2.2
		A12-1.2.1	1/19/2006	1:30 PM	305.62	6.112	3.143	1.94	2.19
		A12-1.2.2	1/19/2006	1:32 PM	248.19	4.964	2.529	1.96	2.21
		A12-1.2.3	1/19/2006	1:33 PM	186.37	3.727	1.894	1.97	2.2
		A12-1.2.4	1/19/2006	1:36 PM	129.63	2.593	1.373	1.89	1.83
		A12-1.2.5	1/19/2006	1:36 PM	157.04	3.141	1.66	1.89	1.91
		A12-1.3.1	1/19/2006	1:38 PM	184.31	3.686	1.925	1.91	1.96
		A12-1.3.2	1/19/2006	1:39 PM	221.81	4.436	2.339	1.9	1.86
		A12-1.3.3	1/19/2006	1:40 PM	214.37	4.287	2.243	1.91	2.02
		A12-1.3.4	1/19/2006	1:41 PM	200.71	4.014	2.116	1.9	1.98
		A12-1.3.5	1/19/2006	1:42 PM	230.52	4.61	2.394	1.93	2.08
√	√	A12-2.1.1	1/20/2006	10:07 PM	171.54	3.431	1.768	1.94	1.97
√	√	A12-2.1.2	1/20/2006	10:07 PM	140.7	2.814	1.452	1.94	1.93
√	√	A12-2.1.3	1/20/2006	10:08 PM	167.74	3.355	1.733	1.94	2
√	√	A12-2.1.4	1/20/2006	10:09 PM	181.74	3.635	1.882	1.93	2
√	√	A12-2.1.5	1/20/2006	10:09 PM	177.06	3.541	1.843	1.92	1.95
		A12-2.2.1	1/19/2006	1:43 PM	219.4	4.388	2.29	1.92	2
		A12-2.2.2	1/19/2006	1:43 PM	189.94	3.799	1.977	1.92	2.26
		A12-2.2.3	1/19/2006	1:44 PM	274.16	5.483	2.85	1.92	2.08
		A12-2.2.4	1/19/2006	1:45 PM	215.81	4.316	2.24	1.93	2.19
		A12-2.2.5	1/19/2006	1:46 PM	220.18	4.404	2.282	1.93	2.12
√	√	A12-2.3.1	1/20/2006	10:10 PM	163.87	3.277	1.695	1.93	1.94
√	√	A12-2.3.2	1/20/2006	10:11 PM	172.63	3.453	1.835	1.88	1.98
√	√	A12-2.3.3	1/20/2006	10:11 PM	169.09	3.382	1.739	1.94	2.02
√	√	A12-2.3.4	1/20/2006	10:12 PM	180.61	3.612	1.872	1.93	1.97
√	√	A12-2.3.5	1/20/2006	10:13 PM	121.52	2.43	1.262	1.93	1.92

		A12-3.1.1	1/19/2006	1:47 PM	136.5	2.73	1.438	1.9	2
		A12-3.1.2	1/19/2006	1:48 PM	203.52	4.07	2.106	1.93	2.18
		A12-3.1.3	1/19/2006	1:49 PM	209.22	4.184	2.189	1.91	1.98
		A12-3.1.4	1/19/2006	1:50 PM	251.47	5.029	2.623	1.92	1.76
		A12-3.1.5	1/19/2006	1:51 PM	222.06	4.441	2.355	1.89	1.63
		A12-3.2.1	1/19/2006	1:52 PM	173.52	3.47	1.807	1.92	1.79
		A12-3.2.2	1/19/2006	1:54 PM	222.17	4.443	2.312	1.92	1.87
		A12-3.2.3	1/19/2006	1:55 PM	261.31	5.226	2.701	1.93	1.92
		A12-3.2.4	1/19/2006	2:01 PM	272.15	5.443	2.782	1.96	2.27
		A12-3.2.5	1/19/2006	2:02 PM	212.19	4.244	2.155	1.97	2.21
		A12-3.3.1	1/19/2006	2:03 PM	159.08	3.182	1.632	1.95	2.06
		A12-3.3.2	1/19/2006	2:04 PM	247.46	4.949	2.523	1.96	2.21
		A12-3.3.3	1/19/2006	2:05 PM	202.85	4.057	2.079	1.95	2.19
		A12-3.3.4	1/19/2006	1:25 PM	133.83	2.677	1.358	1.97	2.18
		A12-3.3.5	1/19/2006	1:25 PM	214.7	4.294	2.218	1.94	2.15

Appendix K. GenomeLab Methods Development Kit Dye Terminator Cycle Sequencing Protocol (Beckman Coulter Inc., Fullerton, CA, 2005)

Materials provided by Beckman Coulter:
Methods Development Kit (P/N 608000):

- DNA polymerase
- Dye Terminators (ddUTP, ddGTP, ddCTP, ddATP)
- dNTP(I) Mix Solution
- dNTP(G) Mix Solution
- Sequencing Reaction Buffer
- pUC18 Control Template (0.25 µg/µL)
- M13 -47 Sequencing Primer (1.6 pmol/µL or 1.6 µM)
- Glycogen (20 mg/mL)
- Mineral Oil
- Sample Loading Solution (SLS)

Required materials not provided by Beckman Coulter:

- Molecular Biology Grade: Sterile dH2O, 95% (v/v) ethanol/dH2O, 70% (v/v) ethanol/ dH2O
- 3M Sodium Acetate pH 5.2 - Sigma, Cat # 430771
- 100 mM Na2-EDTA pH 8.0 (diluted from 0.5M Na2-EDTA pH 8.0 - Sigma, Cat # 7889)
- Sterile tubes, 0.5 mL microfuge, 0.2 mL thin-wall thermal cycling tubes or plates
- Thermal cycler with heated lid

Preparation and Storage
Preparation and Storage of the Kit:
Storage of the Methods Development kit must be in a -20°C non-frost-free freezer.
Preparation and Storage of the Premix:
1. Prepare each Premix in a sterile 1.5 microfuge tube:

Component	dITP Chemistry	dGTP Chemistry
10X Sequencing Reaction Buffer	200 µL	200 µL
dNTP Mix	100 µL	100 µL
ddUTP Dye Terminator	200 µL	200 µL
ddGTP Dye Terminator	**100 µL**	**400 µL**
ddCTP Dye Terminator	200 µL	200 µL
ddATP Dye Terminator	200 µL	200 µL
Polymerase Enzyme	100 µL	100 µL
Total Volume	1100 µL	1400 µL

2. Mix and aliquot the Premix into sterile 0.5 mL microfuge tubes:

Each aliquot is enough for 16 samples.

3. Store the aliquots in a -20°C non-frost-free freezer. Minimize freezing and thawing of the aliquoted Premix.

Preparation of the DNA sequencing reaction*:
Prepare the 20 µL sequencing reaction in a 0.2 mL thin-wall tube or microplate well. Keep all reagents on ice while preparing the sequencing reactions and add components in the order listed below.
.

Component	dITP Chemistry	dGTP Chemistry
H_2O (to adjust total volume to 20 µL)	x.x µL	x.x µL
DNA Template† (See Template Preparation)	0.5 - 7.0 µL	0.5 - 4.0 µL
Customer supplied or -47 Sequencing Primer (1.6 pmol/µL or 1.6µM)	2.0 µL	2.0 µL
Premix	11.0 µL	14.0 µL
Total Volume	20.0 µL	20.0 µL

*Note: Mix reaction components thoroughly. Consolidate the liquid to the bottom of the tube or well by briefly centrifuging before thermal cycling.

Thermal cycling programs:

dITP Chemistry:		dGTP Chemistry:	
96°C	20 sec.	96°C	20 sec.
50°C	20 sec.	50-68°C	20 sec.**
60°C	4 min.	68°C	2 min.

for 30 cycles followed by holding at 4°C

**For the supplied M13 -47 primer, an annealing temperature of 58°C is suitable for most templates. The thermal cycling parameters may need to be modified for other primer and template combinations. For the annealing step, a temperature based on the primer melting temperature (Tm) minus 3 to 5°C is recommended as a starting point.

Ethanol precipitation:
1. Prepare a labeled, sterile 0.5 mL microfuge tube for each sample.
2. Prepare fresh Stop Solution/Glycogen mixture as follows (per sequencing reaction): 2 µL of 3M Sodium Acetate (pH 5.2), 2 µL of 100mM Na2-EDTA (pH 8.0) and 1µL of 20 mg/mL of glycogen (supplied with the kit). To each of the labeled tubes, add 5 µL of the Stop Solution/Glycogen mixture. Transfer the sequencing reaction to the appropriately labeled 0.5 mL tube and mix thoroughly.
3. Add 60 µL cold 95% (v/v) ethanol/dH2O from -20°C freezer and mix thoroughly. Immediately centrifuge at 14,000 rpm at 4°C for 15 minutes. Carefully remove the supernatant with a micropipette (the pellet should be visible).
Note: For multiple samples, always add the cold ethanol/dH2O immediately before centrifugation.
4. Rinse the pellet 2 times with 200 µL 70% (v/v) ethanol/dH2O from -20°C freezer. For each rinse, centrifuge immediately at 14,000 rpm at 4°C for a minimum of 2 minutes. After centrifugation carefully remove all of the supernatant with a micropipette.
5. Vacuum dry for 10 minutes (or until dry).
6. Resuspend the sample in 40 µL of the Sample Loading Solution (provided in the kit). See Appendix C for handling and storage of the Sample Loading Solution.
Note: For plate precipitation instructions, refer to the Applications

Information Bulletin (A1903A), A Rapid and Efficient Method for the Post-Reaction Clean Up of Labeled Dye Terminator Sequencing Products.

Sample preparation for loading into the instrument:

1. Transfer the resuspended samples to the appropriate wells of the polypropylene sample plate recommended for the instrument.
2. Overlay each of the resuspended samples with one drop of light mineral oil (provided in the kit).
3. Load the sample plate into the instrument and start the desired method.

Appendix L. Protocol: Plasmid DNA Purification Using the QIAprep Spin Miniprep Kit and a Microcentrifuge (Qiagen, 2005)

This protocol is designed for purification of up to 20 µg of high-copy plasmid DNA from 1–5 ml overnight cultures of *E. coli* in LB (Luria-Bertani) medium. For purification of low-copy plasmids and cosmids, large plasmids (>10 kb), and DNA prepared using other methods, refer to the recommendations on page 44.

Note: All protocol steps should be carried out at room temperature.

Procedure

1. Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.

Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet. If LyseBlue reagent has been added to Buffer P1, vigorously shake the buffer bottle to ensure LyseBlue particles are completely dissolved. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

2. Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4–6 times.

Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.

If LyseBlue has been added to Buffer P1 the cell suspension will turn blue after addition of Buffer P2. Mixing should result in a homogeneously colored suspension. If the suspension contains localized colorless regions or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

3. Add 350 µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4–6 times.

To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer N3. Large culture volumes (e.g. ≥5 ml) may require inverting up to 10 times. The solution should become cloudy. If LyseBlue reagent has been used, the suspension should be mixed until all trace of blue has gone and the suspension is colorless. A homogeneous colorless suspension indicates that the SDS has been effectively precipitated.

4. Centrifuge for 10 min at 13,000 rpm (~17,900 x *g*) in a table-top microcentrifuge.

A compact white pellet will form.

5. Apply the supernatants from step 4 to the QIAprep spin column by decanting or pipetting.

6. Centrifuge for 30–60 s. Discard the flow-through.

7. Recommended: Wash the QIAprep spin column by adding 0.5 ml Buffer PB and centrifuging for 30–60 s. Discard the flow-through.

This step is necessary to remove trace nuclease activity when using *endA*+ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5〈™ do not require this additional wash step.

8. Wash QIAprep spin column by adding 0.75 ml Buffer PE and centrifuging for 30–60 s.

9. Discard the flow-through, and centrifuge for an additional 1 min to remove residual wash buffer.

Important: Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.

10. Place the QIAprep column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of each QIAprep spin column, let stand for 1 min, and centrifuge for 1 min.

Appendix M. Edited Sequences (FASTA Format)

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TGAGAGGATGATCAGCCACACTGGAACTGAGATACGGTCCAGACTCCTACGGGAGGCAGC
AGTGGGGAATATTGCGCAATGGGCGAAAGCCTGACGCAGCAACGCCGCGTGAGTGATGAA
GGCCCTCGGGTCGTAAAGCTCTGTCGGAGGGAACGAATGTTTCGGGGTTAACAGCCCCGG
GAAGTGACGGTACCCTCAAAGGAAGCCCCGGCTAACTCTGTGCCCGCAGCCCCGG
>A12-3.3.5
AGAGTTTGATCCTGGCTCAGAGTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAAC
GGCAGCACAGCAGTAGCAATACTGTGGGTGGCGAGTGGCGGACGGGTGAGGAATACATCG
GAATCTACCATGTCGTGGGGGATAACGTAGAGGAAACTTACGCTAATACCGACATACGAA
CTATGGTTGAAAGCGGCGGACCGGCAAGGCGTCGCGCGATTGGATGAGCCGATGTACTCG
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GAGAGGATGATCAAGCCAGCACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAG
CAGTGGGGAATATTGGACAATGGGGGCAACCCTGATCCAGCCATGCCGCGTGTGTGAAGA
ATGCCTTCGGGTTGTAAAGCACTTTTGTTCGGGAAGAAAAGCCGCCGGATTAATACCTGT
GTGTCATGACGGTAGCGAGAAGAATAAGCACCGGCTAACTTCGCTGCCAGCAGCC

Appendix N. Isolated Plasmid BLAST Results

Sample	Plasmid Isolation ID	Accession #	Definition	Isolation Source	% Identity	Alignment Length	Mismatches	evalue	Sequence Length	Bit Score	Mesocosm Depth	Planting
A10-1	2.2	AB234243	Uncultured bacterium gene for 16S rRNA, partial sequence, clone:LS4-147	sediment and soil: Japan; Polychlorinated Dioxins	96.17	522	9	0.0	773	843	1	*E. erythropoda*
A10-1	1.3	AJ306790	Uncultured bacterium partial 16S rRNA gene, clone SHA-59	environmental samples	86.71	429	34	3.00E-125	753	457	1	*E. erythropoda*
A10-1	3.5	AM084892	Uncultured Acidobacteria bacterium partial 16S rRNA gene, clone JG35-U3-JT35	soil sample from uranium mining waste	95.15	454	11	0.0	726	706	1	*E. erythropoda*
A10-1	2.1	AY095378	Uncultured yard-trimming-compost bacterium clone S-4 16S ribosomal RNA gene, partial sequence	microbial community of a biofilter-treating hydrogen sulfide and methanol	76.13	419	50	3.00E-45	669	191	1	*E. erythropoda*
A10-1	1.1	AY395143	Uncultured bacterium clone D26ST 16S ribosomal RNA gene, partial sequence	forest soil	90.05	442	28	8.00E-164	704	585	1	*E. erythropoda*
A10-1	2.7	AY493917	Uncultured soil bacterium clone 439 small subunit ribosomal RNA gene, partial sequence	soil	97.49	518	5	0.0	695	878	1	*E. erythropoda*
A10-1	2.4	AY493961	Uncultured soil bacterium clone 1 small subunit ribosomal RNA gene, partial sequence	soil	85.35	471	46	4.00E-128	709	466	1	*E. erythropoda*
A10-1	2.6	AY921798	Uncultured Gemmatimonadetes bacterium clone AKYG1566 16S ribosomal RNA gene, partial sequence	farm soil adjacent to a silage storage bunker	90.22	542	35	0.0	770	691	1	*E. erythropoda*
A10-1	2.3	DQ067029	Uncultured bacterium clone pLW-45 16S ribosomal RNA gene, partial sequence	sediment of Lake Washington	93.79	451	20	0.0	623	645	1	*E. erythropoda*
A10-1	2.5	DQ088264	Uncultured bacterium clone cs47 16S ribosomal RNA gene, partial sequence	Shuangcheng moat sediment	90.91	517	21	0.0	674	671	1	*E. erythropoda*
A10-1	3.3	DQ154365	Uncultured soil bacterium clone RFS-C37 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	90.28	545	37	0.0	736	699	1	*E. erythropoda*
A10-1	3.4	Z85732	Bacterial species 16S rRNA gene	Holophaga / Acidobacterium phylum	96.65	269	6	2.00E-121	747	444	1	*E. erythropoda*
A10-2	1.2	AF293010	Uncultured Green Bay ferromanganous micronodule bacterium MNC2 16S ribosomal RNA gene	freshwater ferromanganous micronodules	89.98	439	3	4.00E-147	580	529	2	*E. erythropoda*
A10-2	2.3	AJ518553	Unidentified bacterium partial 16S rRNA gene, clone QuiP1-81	sediment	94.29	280	12	4.00E-116	448	425	2	*E. erythropoda*
A10-2	2.4	AY150868	Uncultured Rubrobacteridae bacterium clone glen99_21 16S ribosomal RNA gene, partial sequence	disturbed surface soil	92.82	529	30	0.0	692	761	2	*E. erythropoda*
A10-2	3.1	AY214798	Uncultured Acidobacteria bacterium clone BB-2-H5 16S ribosomal RNA gene	soil	98.22	505	8	0.0	751	881	2	*E. erythropoda*
A10-2	2.5	AY568858	Uncultured bacterium isolate JH12_C17 16S ribosomal RNA gene, partial sequence	intertidal flat of Ganghwa Island	86.82	516	43	2.00E-146	679	527	2	*E. erythropoda*
A10-2	2.1	AY592152	Uncultured bacterium clone Kazan-2B-28 BC19-2B-28 16S ribosomal RNA gene, partial sequence	Kazan mud volcano, Eastern Mediterranean, 1673m water depth: isolated from sediment layer 6-22 cm	89.82	226	16	5.00E-73	773	283	2	*E. erythropoda*
A10-2	1.3	AY703473	Uncultured bacterium isolate ALT2 16S ribosomal RNA gene, partial sequence	Altamira Cave	89.33	225	7	5.00E-68	756	267	2	*E. erythropoda*
A10-2	3.3	DQ128807	Uncultured soil bacterium clone HSB NT55_D05 16S ribosomal RNA gene, partial sequence	no tillage soil	97.61	502	7	0.0	730	856	2	*E. erythropoda*
A10-3	1.1	AM158370	Uncultured bacterium partial 16S rRNA gene, clone S408D	Salix rhizosphere in constructed wetland	98.09	471	6	0.0	696	817	3	*E. erythropoda*
A10-3	3.3	AM159274	Uncultured Chloroflexi bacterium 16S rRNA gene, clone HrhB59	rice rhizosphere	88.43	484	47	7.00E-156	726	558	3	*E. erythropoda*
A10-3	3.4	AY395412	Uncultured Acidobacteria bacterium clone EB1093 16S ribosomal RNA gene, partial sequence	pasture soil	87.21	524	44	3.00E-164	720	586	3	*E. erythropoda*
A10-3	3.7	AY464548	Thermomonosporaceae bacterium CNR431 small subunit ribosomal RNA gene, partial sequence	marine sediment	92.13	508	26	0.0	737	704	3	*E. erythropoda*
A10-3	3.5	AY921686	Uncultured Bacteroidetes bacterium clone AKYH1028 16S ribosomal RNA gene, partial sequence	farm soil adjacent to a silage storage bunker	87.31	323	20	5.00E-93	785	350	3	*E. erythropoda*
A10-3	1.5	AY989633	Uncultured soil bacterium clone L1A 15H01 16S ribosomal RNA gene, partial sequence	soil (AK)	96.99	465	10	0.0	671	778	3	*E. erythropoda*
A10-3	2.2	DQ154551	Uncultured soil bacterium clone RFS-C237 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	92.61	487	19	0.0	659	693	3	*E. erythropoda*
A10-3	2.1	DQ154579	Uncultured soil bacterium clone RFS-C265 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	96.24	505	12	0.0	699	822	3	*E. erythropoda*
A10-3	2.4	DQ154600	Uncultured soil bacterium clone RFS-C287 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	97.65	425	10	0.0	518	730	3	*E. erythropoda*

Sample	Plasmid Isolation ID	Accession #	Definition	Isolation Source	% Identity	Alignment Length	Mismatches	evalue	Sequence Length	Bit Score	Mesocosm Depth	Planting
A10-3	2.3	DQ154649	Uncultured soil bacterium clone RFS-C339 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	93.58	530	15	0.0	653	773	3	*E. erythropoda*
A11	1.2	AB091329	Uncultured bacterium gene for 16S rRNA, partial sequence, clone:UI	Methanogenic isophthalate-degrading enrichment culture: Taiwan	90.61	554	26	0.0	662	712	1	*C. comosa*
A11	3.5	AF523332	Uncultured bacterium clone P4-1 16S ribosomal RNA gene, partial sequence	sediment at an inactive uranium mine	99.11	451	4	0.0	765	815	1	*C. comosa*
A11	3.3	AJ583189	uncultured CFB group bacterium partial 16S rRNA gene, clone S15B-MN138	ground water from a monitoring deep-well at a radioactive waste disposal site	93.98	498	23	0.0	719	747	1	*C. comosa*
A11	2.1	AJ585959	Thermococcales archaeon T30a-17 partial 16S rRNA gene	hydrothermal sample in a gas-lift bioreactor	96.36	495	5	0.0	724	806	1	*C. comosa*
A11	2.4	AY212696	Uncultured bacterium clone 248ds10 16S ribosomal RNA gene, partial sequence	water 10 m downstream of equine manure	93.6	516	19	0.0	691	760	1	*C. comosa*
A11	2.3	AY723310	Uncultured epsilon proteobacterium clone P4B-33 16S ribosomal RNA gene, partial sequence	sediment	96.11	489	9	0.0	715	771	1	*C. comosa*
A11	1.1	AY921674	Uncultured delta proteobacterium clone AKYH1085 16S ribosomal RNA gene, partial sequence	farm soil adjacent to a silage storage bunker	96.61	531	15	0.0	719	878	1	*C. comosa*
A11	2.5	AY988694	Uncultured soil bacterium clone L1A.2C08 16S ribosomal RNA gene, partial sequence	soil (AK)	95.36	517	13	0.0	748	811	1	*C. comosa*
A11	3.4	DQ128428	Uncultured soil bacterium clone CWT CU01_D01 16S ribosomal RNA gene, partial sequence	Coweeta forest soil	92.29	519	26	0.0	713	708	1	*C. comosa*
A11	3.1	DQ154525	Uncultured soil bacterium clone RFS-C208 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	97.39	537	6	0.0	667	907	1	*C. comosa*
A11-1	2.3	AB240280	Uncultured bacterium gene for 16S rRNA, partial sequence	PCR-derived sequence from rhizosphere biofilm of reed bed reactor in the laboratory	88.09	470	21	1.00E-143	608	518	1	None
A11-1	3.3	AB240491	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: SRRT63	PCR-derived sequence from root-tip (0 to 40 mm) of Phragmites at Sosei River in Sappro, Japan	87.73	440	40	1.00E-138	794	501	1	None
A11-1	1.6	AF365772	Uncultured bacterium clone BT60DS4BH5 16S ribosomal RNA gene, partial sequence	coral-associated: Panama	93.4	485	23	0.0	719	710	1	None
A11-1	2.4	AY568839	Uncultured bacterium isolate JH10_C91 16S ribosomal RNA gene, partial sequence	intertidal flat of Ganghwa Island	85.16	539	49	5.00E-147	723	529	1	None
A11-1	3.2	AY741700	Uncultured bacterium clone TTMF87 16S ribosomal RNA gene, partial sequence	borehole water from gold mine	91.95	522	38	0.0	726	728	1	None
A11-1	1.3	AY943365	Uncultured soil bacterium clone C05-2 16S ribosomal RNA gene, partial sequence	soil	99.59	483	2	0.0	683	881	1	None
A11-1	1.4	AY989142	Uncultured soil bacterium clone L1A.8F11 16S ribosomal RNA gene, partial sequence	soil (AK)	90.1	505	36	0.0	795	643	1	None
A11-1	3.4	DQ067017	Uncultured bacterium clone pLW-73 16S ribosomal RNA gene, partial sequence	sediment of Lake Washington	96.21	528	17	0.0	695	861	1	None
A11-1	1.1	DQ154336	Uncultured soil bacterium clone RFS-C5 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	96.41	529	15	0.0	785	869	1	None
A11-1	3.5	DQ154499	Uncultured soil bacterium clone RFS-C180 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	87.03	586	8	4.00E-168	737	599	1	None
A11-2	1.4	AB177211	Uncultured bacterium gene for 16S rRNA, partial sequence	PCR-derived sequence from methane hydrate bearing subseafloor sediment at the Peru margin	88.25	332	17	4.00E-102	525	379	2	None
A11-2	3.2	AB240347	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: RB349	PCR-derived sequence from rhizosphere biofilm of reed bed reactor in the laboratory	90.56	286	17	4.00E-99	765	370	2	None
A11-2	2.4	AJ006027	Unidentified eubacterium 16S rRNA gene (clone TBS3)	Soil	96.63	534	12	0.0	684	883	2	None
A11-2	2.1	AJ863284	Uncultured bacterium partial 16S rRNA gene, clone 26BSF49	poplar tree microcosm, bulk soil, flooded	96.46	509	12	0.0	746	835	2	None
A11-2	1.1	AM085466	Uncultured bacterium partial 16S rRNA gene, clone E173	deep sea sediment	91.24	445	24	7.00E-166	723	592	2	None
A11-2	3.4	AM086075	Uncultured bacterium partial 16S rRNA gene, clone c1LKS14	lake profundal sediment: Israel	84.47	528	54	6.00E-137	740	496	2	None
A11-2	1.3	AY193286	Uncultured candidate division OP11 bacterium clone WSA68 16S ribosomal RNA gene, partial sequence	contaminated aquifer	88.15	481	33	1.00E-153	683	551	2	None
A11-2	1.2	AY217482	Uncultured delta proteobacterium clone WCB22 16S ribosomal RNA gene, partial sequence	saturated sediment, Wind Cave, South Dakota	92.66	518	32	0.0	673	743	2	None
A11-2	2.5	AY922118	Uncultured Chloroflexi bacterium clone AKYH1480 16S ribosomal RNA gene, partial sequence	farm soil adjacent to a silage storage bunker	94.41	483	14	0.0	628	713	2	None

Sample	Plasmid Isolation ID	Accession #	Definition	Isolation Source	% Identity	Alignment Length	Mismatches	evalue	Sequence Length	Bit Score	Mesocosm Depth	Planting
A11-2	2.3	DQ125846	Uncultured bacterium clone AKAU4075 16S ribosomal RNA gene, partial sequence	uranium contaminated soil	93.63	455	22	0.0	553	673	2	None
A11-2	3.1	DQ154346	Uncultured soil bacterium clone RFS-C16 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	97.37	533	12	0.0	639	905	2	None
A11-3	3.3	AF293010	Uncultured Green Bay ferromanganous micronodule bacterium MNC2 16S ribosomal RNA gene	freshwater ferromanganous micronodules	99.31	434	1	0.0	718	784	3	None
A11-3	3.5	AF495430	Uncultured eubacterium clone F13.34 16S ribosomal RNA gene, partial sequence	sludge of TaeJon WWTP	96.48	511	12	0.0	683	841	3	None
A11-3	1.2	AY144276	Uncultured Flavobacterium sp. LTUCFB05114 16S ribosomal RNA gene, partial sequence	petroleum-contaminated sandy soil	93.62	470	17	0.0	688	667	3	None
A11-3	2.5	AY150890	Uncultured bacterium clone GR6 16S ribosomal RNA gene, partial sequence	disturbed surface soil	92.65	558	31	0.0	657	795	3	None
A11-3	1.4	AY177763	Uncultured Gram-positive bacterium isolate 5G01 16S ribosomal RNA gene, partial sequence	vadose material found four meters below grassland: CA	95.83	504	12	0.0	636	806	3	None
A11-3	3.4	AY360638	Uncultured myxobacterium clone M10Ba49 small subunit ribosomal RNA gene, partial sequence	oxic rice field soil	93.46	535	26	0.0	682	785	3	None
A11-3	3.1	AY921965	Uncultured Chloroflexi bacterium clone AKYG1623 16S ribosomal RNA gene, partial sequence	farm soil adjacent to a silage storage bunker	90.52	496	32	0.0	646	641	3	None
A11-3	2.4	AY960262	Uncultured beta proteobacterium clone 894 16S ribosomal RNA gene, partial sequence	Altamira Cave wall	97.92	530	8	0.0	634	915	3	None
A11-3	3.2	DQ110128	Uncultured bacterium clone 451T3 16S ribosomal RNA gene, partial sequence	freshwater sediment	93.09	492	18	0.0	702	710	3	None
A12	1.4	AF365844	Uncultured bacterium clone BT60PA10BE6 16S ribosomal RNA gene, partial sequence	coral-associated: Panama	83.92	286	21	3.00E-64	682	254	2	*C. comosa*
A12	2.3	AJ416168	Uncultured bacterium partial 16S rRNA gene, clone Sta0-45	freshwater	95.47	508	10	0.0	753	798	2	*C. comosa*
A12	1.3	AY444993	Uncultured bacterium clone L15 16S ribosomal RNA gene, partial sequence	biofilm on oxygen-transfer membrane	94.04	436	10	0.0	671	647	2	*C. comosa*
A12	2.1	DQ067039	Uncultured bacterium clone pLW-47 16S ribosomal RNA gene, partial sequence	sediment of Lake Washington	87.07	441	45	3.00E-135	783	490	2	*C. comosa*
A12	3.1	DQ110119	Uncultured delta proteobacterium clone 414T3 16S ribosomal RNA gene, partial sequence	freshwater sediment	93.15	409	22	6.00E-161	673	575	2	*C. comosa*
A12	3.2	DQ128773	Uncultured soil bacterium clone HSB NT51_H10 16S ribosomal RNA gene, partial sequence	no tillage soil	84.62	455	23	2.00E-111	717	411	2	*C. comosa*
A12	2.2	DQ154346	Uncultured soil bacterium 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	84.85	549	39	4.00E-137	747	496	2	*C. comosa*
A12	2.5	DQ211455	Uncultured bacterium clone nsc105 16S ribosomal RNA gene, partial sequence	Shimanto River system	85.66	265	15	1.00E-68	655	268	2	*C. comosa*
A12	3.4	DQ310755	Uncultured bacterium clone WCC77B-C01 16S ribosomal RNA gene, partial sequence	riverine sediment	92.05	528	30	0.0	747	732	2	*C. comosa*
A12	1.5	Z95736	Bacterial species 16S rRNA gene (clone mb2431)	Holophaga/Acidobacterium phylum	98.68	532	6	0.0	829	946	2	*C. comosa*
A12-1	1.5	AB240237	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: BS047	PCR-derived sequence from bulk soil of reed bed reactor	94.76	496	22	0.0	703	769	1	*S. atrovirens*
A12-1	2.3	AF243334	Desulforegula conservatrix 16S ribosomal RNA gene, partial sequence	*Desulforegula conservatrix*	93.71	302	4	7.00E-120	525	438	1	*S. atrovirens*
A12-1	3.1	AJ863185	Uncultured bacterium partial 16S rRNA gene, clone 25BSU46	poplar tree microcosm, bulk soil, unflooded	93.14	510	22	0.0	667	736	1	*S. atrovirens*
A12-1	1.2	AY568858	Uncultured bacterium isolate JH12_C17 16S ribosomal RNA gene, partial sequence	intertidal flat of Ganghwa Island	85.94	512	43	2.00E-136	672	494	1	*S. atrovirens*
A12-1	1.1	AY592159	Uncultured bacterium clone Kazan-2B-36/BC19-2B-36 16S ribosomal RNA gene, partial sequence]	Kazan mud volcano, Eastern Mediterranean, 1673m water depth: isolated from sediment layer 6-22 cm	90.53	528	40	0.0	750	689	1	*S. atrovirens*
A12-1	2.4	DQ065033	Uncultured freshwater bacterium clone 965019A11.x1 16S ribosomal RNA gene, partial sequence	freshwater	95.97	397	9	6.00E-180	505	638	1	*S. atrovirens*
A12-1	1.4	DQ066684	Uncultured bacterium clone FOTU12(1-6) 16S ribosomal RNA gene, partial sequence	bioreactor pretreating potable water	97.31	520	10	0.0	700	880	1	*S. atrovirens*
A12-1	3.4	DQ093937	Uncultured bacterium clone ga74 16S ribosomal RNA gene, partial sequence	*Camptotheca acuminata* rhizosphere	88.58	499	33	9.00E-164	606	584	1	*S. atrovirens*
A12-1	3.2	DQ154649	Uncultured soil bacterium clone RFS-C339 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	96.37	523	13	0.0	748	856	1	*S. atrovirens*

Sample	Plasmid Isolation ID	Accession #	Definition	Isolation Source	% Identity	Alignment Length	Mismatches	evalue	Sequence Length	Bit Score	Mesocosm Depth	Planting
A12-2	3.3	AB240264	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: BS122	PCR-derived sequence from bulk soil of reed bed reactor	89.03	237	5	4.00E-71	426	276	2	*S. atrovirens*
A12-2	1.3	AF005016	Nocardioides fulvus 16S ribosomal RNA gene, partial sequence	*Nocardioides fulvus*	96.45	423	7	0.0	539	691	2	*S. atrovirens*
A12-2	1.4	AF432717	Uncultured bacterium clone C17.58WL 16S ribosomal RNA gene, partial sequence	from lodgepole pine rhizosphere soil: British Columbia	96.79	468	10	0.0	599	776	2	*S. atrovirens*
A12-2	1.2	AJ585959	Thermococcales archaeon T30a-17 partial 16S rRNA gene	hydrothermal sample in a gas-lift bioreactor	96.5	543	10	0.0	657	889	2	*S. atrovirens*
A12-2	1.1	AY150900	uncultured Acidobacteriales bacterium clone GR20 16S ribosomal RNA gene, partial sequence	disturbed surface soil	88.63	343	2	7.00E-104	431	385	2	*S. atrovirens*
A12-2	3.2	AY625138	Uncultured bacterium clone I-4 16S ribosomal RNA gene, partial sequence	perchloroethylene-contaminated ground water	91.3	517	36	0.0	642	697	2	*S. atrovirens*
A12-2	1.5	AY921967	Uncultured Bacteroidetes bacterium clone AKYG1650 16S ribosomal RNA gene, partial sequence	farm soil adjacent to a silage storage bunker	96.76	525	14	0.0	725	874	2	*S. atrovirens*
A12-2	2.1	AY922170	Uncultured Chloroflexi bacterium clone AKYH910 16S ribosomal RNA gene, partial sequence	farm soil adjacent to a silage storage bunker	91.39	488	22	0.0	651	652	2	*S. atrovirens*
A12-2	3.1	AY988612	Uncultured soil bacterium clone L1A.1A05 16S ribosomal RNA gene, partial sequence	soil (AK)	91.75	509	26	0.0	602	693	2	*S. atrovirens*
A12-3	3.3	AB186818	Uncultured bacterium gene for 16S rRNA, partial sequence, clone:TSBZ10	uncultured clone from polychlorinated dioxin dechlorinating microcosm	92.18	537	24	0.0	679	743	3	*S. atrovirens*
A12-3	1.2	AB234243	Uncultured bacterium gene for 16S rRNA, partial sequence, clone:LS4-147	sediment and soil: Japan; Polychlorinated Dioxins	95.77	520	13	0.0	712	830	3	*S. atrovirens*
A12-3	1.3	AB240264	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: BS122	PCR-derived sequence from bulk soil of reed bed reactor	98.73	474	5	0.0	570	841	3	*S. atrovirens*
A12-3	3.5	AF418945	Uncultured bacterium clone HTB10 16S ribosomal RNA gene, partial sequence	metal-rich particles from a freshwater reservoir: CO	93.55	543	9	0.0	841	785	3	*S. atrovirens*
A12-3	2.5	AF419683	Uncultured bacterium CS_B017 16S ribosomal RNA gene, partial sequence	hydrothermal sediments in the Guaymas Basin	89.78	460	41	1.00E-163	783	584	3	*S. atrovirens*
A12-3	3.1	AJ232797	Unidentified eubacterium 16S rRNA gene	Isolated from rhizoplane of Trifolium repens	97.47	475	10	0.0	686	809	3	*S. atrovirens*
A12-3	2.2	AJ252653	Agricultural soil bacterium clone SC-I-74, 16S rRNA gene (partial)	agricultural soil bacterium SC-I-74	93	543	29	0.0	729	787	3	*S. atrovirens*
A12-3	2.3	AJ252660	Agricultural soil bacterium clone SC-I-84, 16S rRNA gene (partial)	agricultural soil bacterium SC-I-84	95.7	442	14	0.0	690	706	3	*S. atrovirens*
A12-3	3.4	AJ252685	Rhizosphere soil bacterium clone RSC-II-54, 16S rRNA gene (partial)	rhizosphere soil bacterium RSC-II-54	94.05	538	27	0.0	650	811	3	*S. atrovirens*
A12-3	1.1	DQ404804	Uncultured bacterium clone 661259 16S ribosomal RNA gene, partial sequence	contaminated sediment	98.09	524	6	0.0	633	909	3	*S. atrovirens*
A13	3.8	AB113594	Uncultured actinobacterium gene for 16S rRNA, partial sequence, clone: HAuD-MB3	PCR-derived sequence from subsurface geothermal water	94.51	528	22	0.0	725	808	3	*C. comosa*
A13	2.7	AB240356	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: RB371	PCR-derived sequence from rhizosphere biofilm of reed bed reactor in the laboratory	98.46	455	6	0.0	621	800	3	*C. comosa*
A13	2.8	AF293008	Uncultured Green Bay ferromanganous micronodule bacterium MND4 16S ribosomal RNA gene, partial sequence	uncultured Green Bay ferromanganous micronodule bacterium MND4	92.63	407	17	2.00E-160	518	573	3	*C. comosa*
A13	3.4	AJ005994	Unidentified eubacterium 16S rRNA gene (clone TBS1)	Soil	94.91	491	14	0.0	748	758	3	*C. comosa*
A13	1.8	AY921821	Uncultured beta proteobacterium clone AKYH490 16S ribosomal RNA gene, partial sequence	farm soil adjacent to a silage storage bunker	98.07	518	7	0.0	765	791	3	*C. comosa*
A13	2.6	DQ067022	Uncultured bacterium clone pLW-83 16S ribosomal RNA gene, partial sequence	sediment of Lake Washington	97.15	492	9	0.0	771	826	3	*C. comosa*
A13	3.1	DQ303327	Uncultured Bradyrhizobium sp. clone TM2_7 16S ribosomal RNA gene, partial sequence	soil	93.35	496	4	0.0	736	708	3	*C. comosa*
A13	3.7	DQ444089	Uncultured bacterium clone DS3-9 16S ribosomal RNA gene, partial sequence	Songhuajiang River sediment	95.56	540	14	0.0	726	857	3	*C. comosa*
A13	2.5	DQ444117	Uncultured bacterium clone DS3-37 16S ribosomal RNA gene, partial sequence	Songhuajiang River sediment	94.29	525	17	0.0	751	630	3	*C. comosa*
A21	2.1.1	AJ232853	Unidentified eubacterium 16S rRNA gene (clone LRS29)	rhizosphere soil of Lolium perenne	94.67	469	13	0.0	617	717	1	*C. comosa*
A21	2.1.3	AY037685	Uncultured earthworm cast bacterium clone C030 16S ribosomal RNA gene, partial sequence	uncultured earthworm cast	95.7	442	10	0.0	611	702	1	*C. comosa*

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A21	2.3.1	AY221037	Uncultured bacterium clone CCD4 16S ribosomal RNA gene, partial sequence	sediments collected at Charon's Cascade (KY)	93.84	471	19	0.0	651	701	1	*C. comosa*
A21	2.2.2	AY493939	Uncultured soil bacterium clone 455 small subunit ribosomal RNA gene, partial sequence	soil	93.93	478	19	0.0	644	713	1	*C. comosa*
A21	2.2.5	DQ125726	Uncultured bacterium clone AKAU3824 16S ribosomal RNA gene, partial sequence	uranium contaminated soil	94.95	515	15	0.0	635	797	1	*C. comosa*
A21	2.3.2	DQ154525	Uncultured soil bacterium clone RFS-C208 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	98.33	538	8	0.0	694	942	1	*C. comosa*
A21	2.3.3	DQ154525	Uncultured soil bacterium clone RFS-C208 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	98.51	538	7	0.0	700	948	1	*C. comosa*
A21	2.1.2	DQ297965	Uncultured soil bacterium clone UH1 16S ribosomal RNA gene, partial sequence	hydrocarbon contaminated soil	89.91	545	37	0.0	651	686	1	*C. comosa*
A21	2.2.1	DQ444076	Uncultured bacterium clone PH10-39 16S ribosomal RNA gene, partial sequence	Songhuajiang River sediment	89.76	537	35	0.0	703	673	1	*C. comosa*
A22	2.2.4	AB240280	Uncultured bacterium gene for 16S rRNA, partial sequence	PCR-derived sequence from rhizosphere biofilm of reed bed reactor in the laboratory	98.8	416	4	0.0	517	739	2	*C. comosa*
A22	2.2.1	AF418945	Uncultured bacterium clone HTB10 16S ribosomal RNA gene, partial sequence	metal-rich particles from a freshwater reservoir: CO	95.83	528	15	0.0	737	846	2	*C. comosa*
A22	2.3.3	AY703462	Uncultured bacterium isolate ALT39 16S ribosomal RNA gene, partial sequence	Altamira Cave	92.01	313	18	4.00E-118	593	433	2	*C. comosa*
A22	2.3.4	DQ065033	Uncultured freshwater bacterium clone 965019A11.x1 16S ribosomal RNA gene, partial sequence	freshwater	91.3	299	5	5.00E-105	388	388	2	*C. comosa*
A22	2.2.3	DQ129036	Uncultured soil bacterium clone CWT SM01_B01 16S ribosomal RNA gene, partial sequence	Coweeta forest soil	97.35	453	9	0.0	554	767	2	*C. comosa*
A22	2.3.1	DQ154377	Uncultured soil bacterium clone RFS-C50 16S ribosomal RNA gene, partial sequence	Ross Forest soil	98.61	504	7	0.0	607	894	2	*C. comosa*
A23	2.1.3	AM159235	Uncultured Myxococcales bacterium 16S rRNA gene, clone HrhB18	rice rhizosphere	99.07	535	5	0.0	661	961	3	*C. comosa*
A23	2.1.4	AY188292	Uncultured bacterium clone KD1-11 16S ribosomal RNA gene, partial sequence	environmental sample	87.93	489	51	3.00E-159	708	569	3	*C. comosa*
A23	2.3	AY921917	Uncultured Actinobacteria bacterium clone AKYH901 16S ribosomal RNA gene, partial sequence	farm soil adjacent to a silage storage bunker	95.48	376	8	4.00E-166	479	592	3	*C. comosa*
A23	2.5	DQ083114	Uncultured bacterium clone S34 16S ribosomal RNA gene, partial sequence	soil	85.07	268	9	2.00E-61	753	244	3	*C. comosa*
A23	2.2	Z95736	Bacterial species 16S rRNA gene (clone mb2431)	Holophaga/Acidobacterium phylum	93.53	541	26	0.0	759	800	3	*C. comosa*
A31	2.3.2	AF186418	Uncultured sponge symbiont PAFB14 16S ribosomal RNA gene, partial sequence	specific_host="*Theonella swinhoei*"	96.16	495	15	0.0	686	797	1	None
A31	2.2.5	AY188292	Uncultured bacterium clone KD1-11 16S ribosomal RNA gene, partial sequence	environmental sample	92.31	390	9	8.00E-149	498	534	1	None
A31	2.1.2	AY921569	Uncultured eubacterium clone A30 16S ribosomal RNA gene, partial sequence	rice field soil	88.77	463	31	1.00E-152	582	547	1	None
A31	2.3.3	AY921570	Uncultured eubacterium clone AU31 16S ribosomal RNA gene, partial sequence	rice field soil	92.13	432	23	3.00E-168	541	599	1	None
A31	2.2.2	DQ064969	Uncultured freshwater bacterium clone 965017F03.x1 16S ribosomal RNA gene, partial sequence	freshwater	94.57	534	13	0.0	659	811	1	None
A31	2.2.3	DQ154395	Uncultured soil bacterium clone RFS-C68 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	96.41	501	11	0.0	739	819	1	None
A31	2.1.4	DQ444013	Uncultured bacterium clone PH1-12 16S ribosomal RNA gene, partial sequence	Songhuajiang River sediment	91.56	403	30	3.00E-154	641	553	1	None
A32	2.2.3	AF392739	Uncultured bacterium clone CDF3 16S ribosomal RNA gene, partial sequence	isolated from swiss chard rhizoplane	96.81	533	12	0.0	734	885	2	None
A32	2.1.4	AY037735	Uncultured earthworm cast bacterium clone C132 16S ribosomal RNA gene, partial sequence	soil and earthworm cast	95.31	426	0	0.0	535	658	2	None
A32	2.2.5	AY607161	Uncultured candidate division OP10 bacterium clone X9Ba12 small subunit ribosomal RNA gene, partial sequence	anoxic rice field soil	93.06	504	31	0.0	710	734	2	None
A32	2.3.4	AY631071	Jiangella gansuensis strain YIM 002 16S ribosomal RNA gene, partial sequence	Jiangella gansuensis strain YIM 002	92.55	483	30	0.0	608	688	2	None
A32	2.1.3	AY793656	Uncultured bacterium clone K4_48 16S ribosomal RNA gene, partial sequence	lake sediment	97.71	480	9	0.0	689	824	2	None

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A32	2.1.1	AY921793	Uncultured alpha proteobacterium clone AKYH1282 16S ribosomal RNA gene, partial sequence	farm soil adjacent to a silage storage bunker	98.71	465	4	0.0	674	824	2	None
A32	2.3.2	DQ066684	Uncultured bacterium clone FOTU12(1-6) 16S ribosomal RNA gene, partial sequence	bioreactor pretreating potable water	95.98	523	13	0.0	623	845	2	None
A32	2.3.3	DQ154551	Uncultured soil bacterium clone RFS-C237 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	97.68	517	11	0.0	711	887	2	None
A32	2.2.1	DQ154600	Uncultured soil bacterium clone RFS-C287 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	96.28	537	14	0.0	701	876	2	None
A32	2.2.4	DQ404803	Uncultured bacterium clone 661258 16S ribosomal RNA gene, partial sequence	contaminated sediment	91.58	475	34	0.0	707	651	2	None
A33	2.2.1	AF392781	Uncultured bacterium clone LBB3 16S ribosomal RNA gene, partial sequence	lettuce rhizoplane	85.84	459	44	1.00E-128	782	468	3	None
A33	2.1.2	AY043899	Uncultured actinobacterium clone SMS9.30WL 16S ribosomal RNA gene, partial sequence	forest cut-block mineral soil: British Columbia	95.98	497	12	0.0	772	800	3	None
A33	2.1.4	AY188292	Uncultured bacterium clone KD1-11 16S ribosomal RNA gene, partial sequence	environmental sample	87.65	494	52	4.00E-158	696	566	3	None
A33	2.2.5	AY793654	Uncultured bacterium clone K4_28 16S ribosomal RNA gene, partial sequence	lake sediment	95.87	484	17	0.0	654	780	3	None
A33	2.3.2	DQ154435	Uncultured soil bacterium clone RFS-C109 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	96.15	519	12	0.0	649	841	3	None
A33	1.3	DQ154525	Uncultured soil bacterium clone RFS-C208 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	94.04	537	20	0.0	813	804	3	None
A33	2.3.1	DQ444025	Uncultured bacterium clone PH1-24 16S ribosomal RNA gene, partial sequence	Songhuajiang River sediment	96.27	268	8	1.00E-119	806	438	3	None
A41	3.8	AF432652	Uncultured bacterium clone NMW3.195WL 16S ribosomal RNA gene, partial sequence	forest cut-block mineral soil, British Columbia	88.78	419	32	4.00E-138	689	499	1	*E. erythropoda*
A41	2.7	AF507711	Uncultured soil bacterium clone S098 16S ribosomal RNA gene, partial sequence	pinyon-juniper forest soil	90	460	32	4.00E-163	642	582	1	*E. erythropoda*
A41	3.7	AM159259	Uncultured Chloroflexi bacterium 16S rRNA gene, clone HrhB42	rice rhizosphere	92.86	490	25	0.0	677	702	1	*E. erythropoda*
A41	1.5	AY710492	Uncultured proteobacterium clone SIMO-958 16S ribosomal RNA gene, partial sequence	sediment collected on Oct 17, 2000, Sapelo Island Microbial Observatory Dean Creek Marsh sampling site	84.17	417	31	1.00E-93	769	351	1	*E. erythropoda*
A41	3.6	AY921932	Uncultured planctomycete clone AKYG587 16S ribosomal RNA gene, partial sequence	farm soil adjacent to a silage storage bunker	91.7	482	33	0.0	688	662	1	*E. erythropoda*
A41	3.1	DQ065033	Uncultured freshwater bacterium clone 965019A11.x1 16S ribosomal RNA gene, partial sequence	freshwater	93.57	513	15	0.0	744	750	1	*E. erythropoda*
A41	2.6	DQ110052	Uncultured Verrucomicrobiales bacterium clone 176T36 16S ribosomal RNA gene, partial sequence	uncultured Verrucomicrobiales bacterium	94.84	543	23	0.0	722	843	1	*E. erythropoda*
A41	2.4	DQ128489	Uncultured soil bacterium clone CWT CU03_B06 16S ribosomal RNA gene, partial sequence	Coweeta forest soil	80.17	353	19	2.00E-53	904	219	1	*E. erythropoda*
A41	1.8	DQ154490	Uncultured soil bacterium clone RFS-C170 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	93.7	540	22	0.0	748	798	1	*E. erythropoda*
A41	1.7	DQ154580	Uncultured soil bacterium clone RFS-C266 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	92.92	452	13	0.0	732	641	1	*E. erythropoda*
A41	1.6	DQ154645	Uncultured soil bacterium clone RFS-C335 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	95.86	459	13	0.0	728	737	1	*E. erythropoda*
A42	2.6	AB177205	Uncultured bacterium gene for 16S rRNA	PCR-derived sequence from methane hydrate bearing subseafloor sediment at the Peru margin	82.24	562	55	6.00E-127	664	462	2	*E. erythropoda*
A42	2.3	AF400100	Uncultured bacterium Tb3-J 16S ribosomal RNA gene, partial sequence	Fe(III)-reducing bacteria from the Savannah River sediment	92.13	521	33	0.0	721	732	2	*E. erythropoda*
A42	1.5	AJ232797	Unidentified eubacterium 16S rRNA gene	Isolated from rhizoplane of Trifolium repens	98.74	476	3	0.0	765	843	2	*E. erythropoda*
A42	2.7	AJ252611	Agricultural soil bacterium clone SC-I-7, 16S rRNA gene (partial)	Agricultural soil bacterium clone SC-I-7	96.84	506	15	0.0	722	845	2	*E. erythropoda*
A42	2.8	AJ863242	Uncultured bacterium partial 16S rRNA gene, clone 21BSF23	poplar tree microcosm, bulk soil, flooded	99.23	519	1	0.0	636	933	2	*E. erythropoda*
A42	1.7	AJ876729	Uncultured bacterium partial 16S rRNA gene, clone R1_16	chromium contaminated wastewaters: river sediment	94.48	525	17	0.0	709	798	2	*E. erythropoda*
A42	1.8	AY395111	Uncultured bacterium clone B25R 16S ribosomal RNA gene, partial sequence	forest soil	96.71	426	2	0.0	531	699	2	*E. erythropoda*

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A42	3.7	AY711312	Uncultured bacterium clone SIMO-1946 16S ribosomal RNA gene, partial sequence	sediment 4-5cm collected on Aug 01, 2002, Sapelo Island Microbial Observatory Dean Creek Marsh sampling site	90.51	474	25	6.00E-171	695	608	2	*E. erythropoda*
A42	3.6	AY988827	Uncultured soil bacterium clone L1A.4C02 16S ribosomal RNA gene, partial sequence	soil (AK)	94.49	508	17	0.0	725	773	2	*E. erythropoda*
A42	3.8	DQ154525	Uncultured soil bacterium clone RFS-C208 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	96.89	515	6	0.0	623	856	2	*E. erythropoda*
A42	1.6	DQ444032	Uncultured bacterium clone PH1-31 16S ribosomal RNA gene, partial sequence	Songhuajiang River sediment	98.09	472	3	0.0	724	817	2	*E. erythropoda*
A43	2.3.4	AB240237	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: BS047	PCR-derived sequence from bulk soil of reed bed reactor	90.95	420	26	6.00E-155	520	555	3	*E. erythropoda*
A43	2.2.2	AB240352	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: RB358	PCR-derived sequence from rhizosphere biofilm of reed bed reactor in the laboratory	91.27	527	29	0.0	705	702	3	*E. erythropoda*
A43	2.1.2	AJ518795	Uncultured delta proteobacterium partial 16S rRNA gene, clone JG37-AG-90	uranium mining waste pile near Johanngeorgenstadt, soil sample	96.23	478	9	0.0	664	774	3	*E. erythropoda*
A43	2.3.1	AY095430	Uncultured yard-trimming-compost bacterium clone S-67 16S ribosomal RNA gene, partial sequence	yard-trimming-compost	95.42	459	14	0.0	592	725	3	*E. erythropoda*
A43	2.1.5	AY150879	Uncultured Rubrobacteridae bacterium clone glen99_17 16S ribosomal RNA gene, partial sequence	disturbed surface soil	91.12	439	33	3.00E-165	775	590	3	*E. erythropoda*
A43	2.2.5	AY193195	Uncultured candidate division OD1 bacterium clone SRC3 16S ribosomal RNA gene, partial sequence	sulphur river filaments	86.91	489	39	7.00E-146	682	525	3	*E. erythropoda*
A43	2.3.3	DQ154470	Uncultured soil bacterium clone RFS-C148 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	96.45	535	15	0.0	720	880	3	*E. erythropoda*
A43	2.3.2	DQ154548	Uncultured soil bacterium clone RFS-C233 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	90.62	533	38	0.0	749	697	3	*E. erythropoda*
A43	2.3.5	X68461	Unknown Actinomycete (MC 64) 16S rRNA	soil sample from subtropical Australia	92.86	434	31	1.00E-177	737	630	3	*E. erythropoda*
A51	3.6	AF010003	Unidentified eubacterium 16S ribosomal RNA gene, partial sequence	soil	96.07	484	10	0.0	764	780	1	*S. atrovirens*
A51	3.8	AF078361	Grassland soil clone sl1_224 16S ribosomal RNA gene, partial sequence	improved upland grass pasture	92.76	387	22	9.00E-155	756	555	1	*S. atrovirens*
A51	2.3	AF443586	Uncultured bacterium clone C-F-15 16S ribosomal RNA gene, partial sequence	cells extracted from a semiarid soil	92.45	530	26	0.0	653	745	1	*S. atrovirens*
A51	2.2	AJ876725	Uncultured bacterium partial 16S rRNA gene, clone R1_7	chromium contaminated wastewaters: river sediment	91.11	225	10	5.00E-77	624	296	1	*S. atrovirens*
A51	2.7	AY692042	Uncultured Desulfuromonas sp. clone M76 16S ribosomal RNA gene, partial sequence	UASB reactor	93.32	464	20	0.0	670	675	1	*S. atrovirens*
A51	1.1	DQ088236	Uncultured bacterium clone cs11 16S ribosomal RNA gene, partial sequence	Shuangcheng moat sediment	91.15	486	28	0.0	760	645	1	*S. atrovirens*
A51	2.8	DQ129102	Uncultured soil bacterium clone CWT SM03_A11 16S ribosomal RNA gene, partial sequence	Coweeta forest soil	86.45	524	49	8.00E-155	632	555	1	*S. atrovirens*
A51	2.5	DQ154387	Uncultured soil bacterium clone RFS-C60 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	97.7	434	8	0.0	720	745	1	*S. atrovirens*
A51	1.4	DQ154526	Uncultured soil bacterium clone RFS-C209 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	93.41	349	6	8.00E-139	517	501	1	*S. atrovirens*
A51	2.6	DQ154551	Uncultured soil bacterium clone RFS-C237 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	95.7	442	5	0.0	755	699	1	*S. atrovirens*
A51	3.1	DQ154568	Uncultured soil bacterium clone RFS-C254 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	93.19	543	25	0.0	683	791	1	*S. atrovirens*
A51	1.5	DQ195654	Uncultured bacterium isolate High.2.45.F10.HB35 16S ribosomal RNA gene, partial sequence	soil	95.66	438	7	0.0	584	693	1	*S. atrovirens*
A52	2.3	AJ863210	Uncultured bacterium partial 16S rRNA gene, clone 20BSU7	poplar tree microcosm, bulk soil, unflooded	97.08	514	15	0.0	744	867	2	*S. atrovirens*
A52	3.4	AM162457	Uncultured bacterium partial 16S rRNA gene, clone B23	sphagnum peat bog: Russia	94.58	554	8	0.0	706	837	2	*S. atrovirens*
A52	2.5	AY212696	Uncultured bacterium clone 248ds10 16S ribosomal RNA gene, partial sequence	water 10 m downstream of equine manure	88.93	551	23	0.0	730	645	2	*S. atrovirens*
A52	3.2	AY568858	Uncultured bacterium isolate JH12_C17 16S ribosomal RNA gene, partial sequence	intertidal flat of Ganghwa Island	85.96	520	43	4.00E-139	797	503	2	*S. atrovirens*
A52	1.5	AY921932	Uncultured planctomycete clone AKYG587 16S ribosomal RNA gene, partial sequence	farm soil adjacent to a silage storage bunker	93.19	470	20	0.0	683	680	2	*S. atrovirens*

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A52	2.2	DQ093916	Uncultured bacterium clone ga48 16S ribosomal RNA gene, partial sequence	*Camptotheca acuminata* rhizosphere	94.46	433	17	0.0	710	660	2	*S. atrovirens*
A52	1.3	DQ128797	Uncultured soil bacterium clone HSB NT53_C06 16S ribosomal RNA gene, partial sequence	no tillage soil	87.56	442	43	1.00E-138	777	501	2	*S. atrovirens*
A52	1.2	DQ154435	Uncultured soil bacterium clone RFS-C109 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	99.42	521	2	0.0	780	944	2	*S. atrovirens*
A52	3.3	DQ154570	Uncultured soil bacterium clone RFS-C256 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	93.83	519	11	0.0	829	761	2	*S. atrovirens*
A53	1.5	AB187888	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: Niitsu18-45	PCR-derived sequence from compost	93.33	510	28	0.0	611	749	3	*S. atrovirens*
A53	2.2	AY221082	Uncultured bacterium clone CCU5 16S ribosomal RNA gene, partial sequence	sediments collected at Charon's Cascade (KY)	95.09	530	9	0.0	653	821	3	*S. atrovirens*
A53	1.3	AY360642	Uncultured Methylobacteriaceae bacterium clone M10Ba54 small subunit ribosomal RNA gene, partial sequence	oxic rice field soil	96.37	468	16	0.0	743	769	3	*S. atrovirens*
A53	2.3	AY607137	Uncultured candidate division OP10 bacterium clone X3Ba47 small subunit ribosomal RNA gene	anoxic rice field soil	90.94	508	36	0.0	691	675	3	*S. atrovirens*
A53	2.1	AY917698	Uncultured bacterium clone 1959b-39 16S ribosomal RNA gene, partial sequence	volcanic deposit from 1959	93.56	435	13	8.00E-179	542	634	3	*S. atrovirens*
A53	1.2	AY921743	Uncultured Nitrospirae bacterium clone AKYG586 16S ribosomal RNA gene, partial sequence	farm soil adjacent to a silage storage bunker	98.65	520	6	0.0	637	920	3	*S. atrovirens*
A53	3.1	DQ093909	Uncultured bacterium clone ga40 16S ribosomal RNA gene, partial sequence	*Camptotheca acuminata* rhizosphere	94.02	468	19	0.0	650	701	3	*S. atrovirens*
A53	3.4	DQ093937	Uncultured bacterium clone ga74 16S ribosomal RNA gene, partial sequence	*Camptotheca acuminata* rhizosphere	88.98	499	38	1.00E-168	828	601	3	*S. atrovirens*
A53	3.3	DQ125726	Uncultured bacterium clone AKAU3824 16S ribosomal RNA gene, partial sequence	uranium contaminated soil	93.41	455	22	0.0	680	667	3	*S. atrovirens*
A53	3.5	DQ154633	Uncultured soil bacterium clone RFS-C321 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	92.37	498	17	0.0	603	689	3	*S. atrovirens*
A61	2.5	AM086162	Uncultured bacterium partial 16S rRNA gene, clone C5lks103	lake profundal sediment	94.11	492	17	0.0	728	739	1	*S. atrovirens*
A61	1.3	AY324518	Uncultured planctomycete clone GoM_5268WB-14 16S ribosomal RNA, partial sequence	Gulf of Mexico cold seep sediment associated with Beggiatoa microbial mat communities	89.9	307	28	5.00E-106	538	392	1	*S. atrovirens*
A61	1.1	AY360629	Uncultured Chloroflexi bacterium clone M10Ba39 small subunit ribosomal RNA gene, partial sequence	oxic rice field soil	87.19	398	31	9.00E-120	700	438	1	*S. atrovirens*
A61	2.2	AY703470	Uncultured bacterium isolate ALT5 16S ribosomal RNA gene, partial sequence	Altamira Cave	93.42	532	18	0.0	726	773	1	*S. atrovirens*
A61	1.5	AY834348	Uncultured bacterium clone cloRDL-22 16S ribosomal RNA gene, partial sequence	potato rhizosphere	93.5	431	21	1.00E-178	701	634	1	*S. atrovirens*
A61	1.2	AY921533	Uncultured eubacterium clone JU26 16S ribosomal RNA gene, partial sequence	rice field soil	91.12	507	29	4.00E-168	758	599	1	*S. atrovirens*
A61	1.4	AY921859	Uncultured Gemmatimonadetes bacterium clone AKYH1258 16S ribosomal RNA gene, partial sequence	farm soil adjacent to a silage storage bunker	91.46	515	22	0.0	705	688	1	*S. atrovirens*
A61	3.5	DQ154574	Uncultured soil bacterium clone RFS-C260 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	90.36	415	24	1.00E-147	550	531	1	*S. atrovirens*
A61	2.1	DQ154633	Uncultured soil bacterium clone RFS-C321 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	94.22	450	6	0.0	620	669	1	*S. atrovirens*
A62	3.1	AB234243	Uncultured bacterium gene for 16S rRNA, partial sequence, clone:LS4-147	sediment and soil: Japan; Polychlorinated Dioxins	92.39	539	14	0.0	774	743	2	*S. atrovirens*
A62	1.5	AM085466	Uncultured bacterium partial 16S rRNA gene, clone E173	deep sea sediment	97.74	532	12	0.0	758	917	2	*S. atrovirens*
A62	2.3	AY177760	Uncultured Acidobacteria bacterium isolate 5g10 16S ribosomal RNA gene, partial sequence	subsurface soil beneath grassland	94.05	521	19	0.0	649	780	2	*S. atrovirens*
A62	2.4	AY921748	Uncultured delta proteobacterium clone AKYH800 16S ribosomal RNA gene, partial sequence	farm soil adjacent to a silage storage bunker	94.01	534	25	0.0	677	802	2	*S. atrovirens*
A62	3.2	CR933082	Uncultured bacterium partial 16S rRNA gene from clone 030D10_P_DI_P15	anaerobic sludge digester	86.21	319	7	6.00E-82	743	313	2	*S. atrovirens*
A62	3.5	DQ017918	Uncultured bacterium clone W-Btb7_19 16S ribosomal RNA gene, partial sequence	upland stream	94.9	510	16	0.0	618	789	2	*S. atrovirens*
A62	1.4	DQ093906	Uncultured bacterium clone ga37 16S ribosomal RNA gene, partial sequence	*Camptotheca acuminata* rhizosphere	94.51	546	16	0.0	670	830	2	*S. atrovirens*

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A62	1.2	DQ447305	Uncultured bacterium clone LS5 16S ribosomal RNA gene, partial sequence	pulp sediments from paper mill	89.54	526	25	0.0	695	643	2	*S. atrovirens*
A63	2.2	AB240271	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: BS137	PCR-derived sequence from bulk soil of reed bed reactor	97.39	535	5	0.0	640	905	3	*S. atrovirens*
A63	1.3	AJ252663	Agricultural soil bacterium clone SC-I-87, 16S rRNA gene (partial)	agricultural soil bacterium SC-I-87	98.46	520	3	0.0	752	913	3	*S. atrovirens*
A63	3.5	AY123804	Nitrosospira sp. Nsp17 16S ribosomal RNA gene, partial sequence	*Nitrosospira sp. Nsp17*	99.8	500	1	0.0	691	918	3	*S. atrovirens*
A63	3.2	AY197424	Uncultured bacterium clone B01R003 16S ribosomal RNA gene, partial sequence	Guaymas Basin hydrothermal vent sediments	86.71	519	46	7.00E-156	744	558	3	*S. atrovirens*
A63	1.4	AY215232	Mycobacterium celatum isolate N1798T 16S ribosomal RNA gene, partial sequence	*Mycobacterium celatum*	91.48	528	27	0.0	724	710	3	*S. atrovirens*
A63	2.3	AY570628	Uncultured bacterium clone PL-37B10 16S ribosomal RNA gene, partial sequence	low-temperature biodegraded Canadian oil reservoir	91.97	473	19	0.0	775	645	3	*S. atrovirens*
A63	2.4	AY921916	Uncultured alpha proteobacterium clone AKYG1580 16S ribosomal RNA gene, partial sequence	farm soil adjacent to a silage storage bunker	90.51	390	31	2.00E-141	719	510	3	*S. atrovirens*
A63	2.5	DQ154336	Uncultured soil bacterium clone RFS-C5 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	93.45	473	13	0.0	586	686	3	*S. atrovirens*
A63	3.4	DQ154525	Uncultured soil bacterium clone RFS-C208 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	92.72	536	21	0.0	651	758	3	*S. atrovirens*
A63	1.5	DQ154592	Uncultured soil bacterium clone RFS-C278 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	81.82	264	11	3.00E-44	557	187	3	*S. atrovirens*
A71	2.2	AB239707	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: BS007	PCR-derived sequence from bulk soil of reed bed reactor	86.13	548	44	2.00E-164	778	587	1	*E. erythropoda*
A71	2.4	AF050593	Uncultured eubacterium WCHB1-02 16S ribosomal RNA gene	chlorinated-solvent-contaminated aquifer	97.12	452	11	0.0	692	798	1	*E. erythropoda*
A71	NC	AJ585959	Thermococcales archaeon T30a-17 partial 16S rRNA gene	hydrothermal sample in a gas-lift bioreactor	93.07	361	7	1.00E-148	812	535	1	*E. erythropoda*
A71	3.4	AJ966601	Uncultured bacterium partial 16S rRNA gene, clone E60	deep sea sediment	86.65	382	32	1.00E-114	661	421	1	*E. erythropoda*
A71	2.5	AY214626	Uncultured delta proteobacterium clone SL2-1-E7 16S ribosomal RNA gene	soil	86.84	471	28	2.00E-146	654	527	1	*E. erythropoda*
A71	1.3	AY221044	Uncultured bacterium clone CCM12b 16S ribosomal RNA gene	sediments collected at Charon's Cascade (KY)	89.47	532	34	0.0	730	669	1	*E. erythropoda*
A71	2.1	AY268244	Uncultured bacterium clone E9 16S small subunit ribosomal RNA gene	shower curtain biofilm	95.97	472	11	0.0	699	794	1	*E. erythropoda*
A71	1.5	AY869683	Uncultured bacterium clone FS117-41B-02 16S ribosomal RNA gene, partial sequence	ridge flank crustal fluids	84.41	526	49	3.00E-134	718	486	1	*E. erythropoda*
A71	3.5	AY917298	Uncultured bacterium clone 1700a2-32 16S ribosomal RNA gene	volcanic deposit from 1700 (HI)	92.48	479	17	0.0	741	694	1	*E. erythropoda*
A71	2.3	AY921931	Uncultured Gemmatimonadetes bacterium clone AKYG1678 16S ribosomal RNA gene	farm soil adjacent to a silage storage bunker	93.89	491	22	0.0	817	763	1	*E. erythropoda*
A71	3.3	AY921978	Uncultured alpha proteobacterium clone AKYG1729 16S rRNA	farm soil adjacent to a silage storage bunker	98.93	467	2	0.0	728	865	1	*E. erythropoda*
A71	1.4	AY988610	Uncultured soil bacterium clone L1A.1A03 16S ribosomal RNA gene	soil (AK)	94.4	411	17	0.0	803	652	1	*E. erythropoda*
A71	3.1	AY989180	Uncultured soil bacterium clone L1A.9C06 16S ribosomal RNA gene	soil (AK)	92.58	431	8	7.00E-175	624	621	1	*E. erythropoda*
A71	1.1	DQ154377	Uncultured soil bacterium clone RFS-C50 16S ribosomal RNA gene	Ross Forest Soil	94.62	520	8	0.0	718	821	1	*E. erythropoda*
A71	3.2	DQ369352	Uncultured bacterium clone ACE-GEN-49 16S ribosomal RNA gene	freshwater sediment	92	514	17	0.0	712	726	1	*E. erythropoda*
A72	2.5	AB187912	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: Niitsu24-7	PCR-derived sequence from compost	94	533	21	0.0	765	829	2	*E. erythropoda*
A72	1.1	AF317770	Unidentified bacterium wb1_H11 small subunit ribosomal RNA gene, partial sequence	Nullarbor caves, Australia	90.09	545	29	0.0	712	712	2	*E. erythropoda*
A72	3.1	AF392637	Uncultured bacterium clone SCA10 16S ribosomal RNA gene	soybean rhizoplane	95.43	525	13	0.0	765	826	2	*E. erythropoda*
A72	1.4	AJ863212	Uncultured bacterium partial 16S rRNA gene, clone 20BSU45	poplar tree microcosm, bulk soil, unflooded	91.82	501	12	0.0	678	698	2	*E. erythropoda*
A72	2.4	AJ863222	Uncultured bacterium partial 16S rRNA gene, clone 25BSU14	poplar tree microcosm, bulk soil, unflooded	99.56	450	1	0.0	656	852	2	*E. erythropoda*

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A72	2.3	AY212589	Uncultured bacterium clone 139ds10 16S ribosomal RNA gene, partial sequence	water 10 m downstream of equine manure	92.29	493	26	0.0	704	723	2	*E. erythropoda*
A72	1.5	AY214798	Uncultured Acidobacteria bacterium clone BB-2-H5 16S ribosomal RNA gene	soil	94.62	502	6	0.0	702	788	2	*E. erythropoda*
A72	1.2	DQ154362	Uncultured soil bacterium clone RFS-C34 16S ribosomal RNA gene	Ross Forest Soil	89.23	492	33	8.00E-175	707	621	2	*E. erythropoda*
A72	3.4	DQ154364	Uncultured soil bacterium clone RFS-C36 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	92.4	526	35	0.0	765	745	2	*E. erythropoda*
A72	2.2	DQ154525	Uncultured soil bacterium clone RFS-C208 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	91.26	492	21	0.0	682	681	2	*E. erythropoda*
A73	3.2	AF419683	Uncultured bacterium CS_B017 16S ribosomal RNA gene, partial sequence	hydrothermal sediments in the Guaymas Basin	88.34	463	41	2.00E-151	693	544	3	*E. erythropoda*
A73	2.4	AJ863216	Uncultured bacterium partial 16S rRNA gene, clone 20BSU60	poplar tree microcosm, bulk soil, unflooded	95.88	510	12	0.0	752	850	3	*E. erythropoda*
A73	1.4	AM167966	Uncultured candidate division OP11 bacterium partial 16S rRNA gene	bacterial flora in mineral waters	86.67	390	38	2.00E-120	788	440	3	*E. erythropoda*
A73	3.4	AY360666	Uncultured Acidobacteria bacterium clone M10Ba79 small subunit ribosomal RNA gene, partial sequence	oxic rice field soil	90.93	496	14	0.0	722	665	3	*E. erythropoda*
A73	1.3	AY493918	Uncultured soil bacterium clone 199 small subunit ribosomal RNA gene	soil	84.09	509	37	2.00E-128	722	467	3	*E. erythropoda*
A73	2.5	AY568858	Uncultured bacterium isolate JH12_C17 16S ribosomal RNA gene, partial sequence	intertidal flat of Ganghwa Island	82.91	515	47	7.00E-113	742	415	3	*E. erythropoda*
A73	3.3	AY752750	Uncultured Geobacter sp. clone Fe_P2-20 16S ribosomal RNA gene, partial sequence	Banisveld landfill iron-reducing leachate-polluted groundwater	93.86	456	13	0.0	720	673	3	*E. erythropoda*
A73	2.3	DQ125856	Uncultured bacterium clone AKAU4089 16S ribosomal RNA gene, partial sequence	uranium contaminated soil	97.27	476	2	0.0	648	829	3	*E. erythropoda*
A73	2.2	DQ154435	Uncultured soil bacterium clone RFS-C109 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	93.95	479	10	0.0	673	737	3	*E. erythropoda*
A73	1.1	DQ297986	Uncultured soil bacterium clone UC8 16S ribosomal RNA gene, partial sequence	hydrocarbon contaminated soil	86.96	460	24	5.00E-136	729	492	3	*E. erythropoda*
A81	3.5	AB177307	Uncultured bacterium gene for 16S rRNA, partial sequence	PCR-derived sequence from methane hydrate bearing subseafloor sediment at the Cascadia margin	86.72	482	32	9.00E-149	734	535	1	None
A81	1.1	AF392740	Uncultured bacterium clone LAC1 16S ribosomal RNA gene	lettuce rhizoplane	95.84	505	6	0.0	731	835	1	None
A81	1.3	AF392740	Uncultured bacterium clone LAC1 16S ribosomal RNA gene	lettuce rhizoplane	95.94	517	6	0.0	737	858	1	None
A81	3.4	AF524016	Uncultured bacterium clone FW98 16S ribosomal RNA gene, partial sequence	forested wetland	90.7	430	26	2.00E-156	741	560	1	None
A81	1.4	AY043821	Uncultured delta proteobacterium clone SMS9.119WL 16S ribosomal RNA gene, partial sequence	Forest cut-block mineral soil: British Columbia	93.47	291	17	2.00E-117	758	431	1	None
A81	1.5	AY214734	Uncultured Verrucomicrobia bacterium clone BB-1-H5 16S ribosomal RNA gene, partial sequence	Soil	95.25	526	11	0.0	735	858	1	None
A81	2.2	AY596155	Uncultured bacterium clone ISCB-72 16S ribosomal RNA gene, partial sequence	nitrophenol contaminated soil	93.85	455	13	0.0	683	671	1	None
A81	3.2	DQ093888	Uncultured bacterium clone ga16 16S ribosomal RNA gene, partial sequence	*Camptotheca acuminata* rhizosphere	84.34	479	34	1.00E-123	758	451	1	None
A81	1.2	DQ110112	Uncultured bacterium clone 465T3 16S ribosomal RNA gene, partial sequence	freshwater sediment	90.06	503	27	0.0	808	656	1	None
A82	1.5	AB062678	Bacillus sp. MK03 gene for 16S rRNA	Bacillus sp. MK03	95.34	536	14	0.0	704	841	2	None
A82	2.5	AB240276	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: BS152	PCR-derived sequence from bulk soil of reed bed reactor	93.27	520	21	0.0	781	754	2	None
A82	3.5	AJ252662	Agricultural soil bacterium clone SC-I-86, 16S rRNA gene (partial)	agricultural soil	96.82	534	12	0.0	782	887	2	None
A82	1.1	AY154600	Uncultured earthworm cast bacterium clone c245 16S ribosomal RNA gene, partial sequence	earthworm cast	94.28	297	17	9.00E-125	747	455	2	None
A82	3.2	AY921821	Uncultured beta proteobacterium clone AKYH490 16S ribosomal RNA gene	farm soil adjacent to a silage storage bunker	90.51	527	22	0.0	759	671	2	None
A82	3.4	AY921821	Uncultured beta proteobacterium clone AKYH490 16S ribosomal RNA gene	farm soil adjacent to a silage storage bunker	96.69	513	12	0.0	754	848	2	None
A82	1.4	AY922056	Uncultured Chloroflexi bacterium clone AKYG705 16S ribosomal RNA gene, partial sequence	farm soil adjacent to a silage storage bunker	85.07	489	41	3.00E-129	733	470	2	None

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A82	2.4	DQ065033	Uncultured freshwater bacterium clone 965019A11.x1 16S ribosomal RNA gene, partial sequence	freshwater	91.14	519	18	0.0	697	706	2	None
A82	3.1	DQ154571	Uncultured soil bacterium clone RFS-C257 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	88.39	508	39	5.00E-167	735	595	2	None
A82	1.2	DQ154627	Uncultured soil bacterium clone RFS-C314 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	98.14	537	4	0.0	798	931	2	None
A83	1.1	AF423211	Uncultured soil bacterium clone 1152-2 16S ribosomal RNA gene, partial sequence	soil	88.07	436	20	1.00E-138	604	501	3	None
A83	1.4	AJ532704	Uncultured alpha proteobacterium partial 16S rRNA gene, clone JG34-KF-245	uranium mining waste pile	98.05	411	3	0.0	656	710	3	None
A83	3.2	AJ863276	Uncultured bacterium partial 16S rRNA gene, clone 26BSF32	poplar tree microcosm, bulk soil, flooded	98.7	231	1	3.00E-111	346	409	3	None
A83	1.3	AY037566	Uncultured soil bacterium clone S017 16S ribosomal RNA gene, partial sequence	soil and earthworm cast	89.4	481	36	7.00E-166	729	592	3	None
A83	1.2	AY711648	Uncultured bacterium clone SIMO-2282 16S ribosomal RNA gene, partial sequence	sediment 14-16cm collected, Sapelo Island Microbial Observatory Dean Creek Marsh sampling site	91.05	458	25	7.00E-171	765	608	3	None
A83	3.4	AY921920	Uncultured beta proteobacterium clone AKYH951 16S ribosomal RNA gene, partial sequence	farm soil adjacent to a silage storage bunker	97.87	517	11	0.0	673	894	3	None
A83	2.5	AY922099	Uncultured Acidobacteria bacterium clone AKYH1308 16S ribosomal RNA gene, partial sequence	farm soil adjacent to a silage storage bunker	94.59	536	21	0.0	784	822	3	None
A83	3.3	DQ066684	Uncultured bacterium clone FOTU12(1-6) 16S ribosomal RNA gene, partial sequence	bioreactor pretreating potable water	82.47	462	31	1.00E-88	653	335	3	None
A83	2.2	DQ128428	Uncultured soil bacterium clone CWT CU01_D01 16S ribosomal RNA gene, partial sequence	Coweeta forest soil	94.96	516	20	0.0	758	806	3	None
A83	2.4	DQ154627	Uncultured soil bacterium clone RFS-C314 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	90.94	508	18	0.0	677	660	3	None
A91	3.1	AF432674	Uncultured bacterium clone NMS8.35WL 16S ribosomal RNA gene, partial sequence	forest cut-block mineral soil, British Columbia	88.81	536	17	3.00E-174	744	619	1	*S. atrovirens*
A91	1.4	AJ306790	Uncultured bacterium partial 16S rRNA gene, clone SHA-59	environmental samples	84.46	354	41	2.00E-90	665	340	1	*S. atrovirens*
A91	1.3	AJ519650	Uncultured bacterium partial 16S rRNA gene, clone Sh765B-TzT-35	uranium mill tailings, soil sample	96.45	423	10	0.0	560	693	1	*S. atrovirens*
A91	1.5	AJ863262	Uncultured bacterium partial 16S rRNA gene, clone 26BSF28	poplar tree microcosm, bulk soil, flooded	83.28	335	28	6.00E-73	882	283	1	*S. atrovirens*
A91	1.1	AY102911	Uncultured bacterium clone BCM3S-5B 16S ribosomal RNA gene, partial sequence	subtropical freshwater marsh	94.65	523	7	0.0	700	793	1	*S. atrovirens*
A91	1.2	AY102911	Uncultured bacterium clone BCM3S-5B 16S ribosomal RNA gene, partial sequence	subtropical freshwater marsh	96.24	505	12	0.0	698	822	1	*S. atrovirens*
A91	2.4	AY568858	Uncultured bacterium isolate JH12_C17 16S ribosomal RNA gene, partial sequence	intertidal flat of Ganghwa Island	86.6	515	41	1.00E-143	746	518	1	*S. atrovirens*
A91	3.2	DQ125571	Uncultured bacterium clone AKAU3564 16S ribosomal RNA gene, partial sequence	uranium contaminated soil	82.33	215	28	6.00E-42	653	180	1	*S. atrovirens*
A91	2.1	DQ154358	Uncultured soil bacterium clone RFS-C30 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	86.21	551	26	3.00E-154	708	553	1	*S. atrovirens*
A91	3.3	DQ154451	Uncultured soil bacterium clone RFS-C128 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	98.27	521	3	0.0	788	907	1	*S. atrovirens*
A92	2.1	AF407406	Uncultured bacterium clone RA13C7 16S ribosomal RNA gene, partial sequence	reactor system treating monochlorobenzene contaminated groundwater	90.5	505	27	0.0	720	647	2	*S. atrovirens*
A92	2.4	AJ292595	uncultured eubacterium WD233 partial 16S rRNA gene, clone WD233	uncultured eubacterium WD233	98.07	466	9	0.0	664	811	2	*S. atrovirens*
A92	3.4	AJ863208	Uncultured bacterium partial 16S rRNA gene, clone 25BSU20	poplar tree microcosm, bulk soil, unflooded	87.55	273	15	2.00E-77	767	298	2	*S. atrovirens*
A92	3.5	AY177758	Uncultured Acidobacteria bacterium isolate OF20 16S ribosomal RNA gene, partial sequence	grassland soil: CA	97.93	531	11	0.0	775	924	2	*S. atrovirens*
A92	1.2	AY703463	Uncultured bacterium isolate ALT39 16S ribosomal RNA gene, partial sequence	Altamira Cave	95.58	498	12	0.0	644	795	2	*S. atrovirens*
A92	1.3	AY921821	Uncultured beta proteobacterium clone AKYH490 16S ribosomal RNA gene	farm soil adjacent to a silage storage bunker	98.46	518	5	0.0	660	909	2	*S. atrovirens*

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A92	2.3	DQ154350	Uncultured soil bacterium clone RFS-C21 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	97.5	521	9	0.0	707	887	2	*S. atrovirens*
A92	3.3	DQ154584	Uncultured soil bacterium clone RFS-C270 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	95.93	442	15	0.0	567	713	2	*S. atrovirens*
A92	2.2	DQ154617	Uncultured soil bacterium clone RFS-C303 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	97.89	521	3	0.0	657	894	2	*S. atrovirens*
A92	3.2	DQ297974	Uncultured soil bacterium clone UA11 16S ribosomal RNA gene, partial sequence	hydrocarbon contaminated soil	88.73	550	41	0.0	738	652	2	*S. atrovirens*
A93	1.1	AB240310	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: RB127	PCR-derived sequence from rhizosphere biofilm of reed bed reactor in the laboratory	94.63	540	20	0.0	780	828	3	*S. atrovirens*
A93	2.5	AJ863223	Uncultured bacterium partial 16S rRNA gene, clone 25BSU19	poplar tree microcosm, bulk soil, unflooded	94.31	492	11	0.0	639	737	3	*S. atrovirens*
A93	3.5	AY242757	Uncultured soil bacterium clone Tc135-226 16S ribosomal RNA gene, partial sequence	heavy metal-contaminated bulk and rhizosphere soil	93.84	422	22	3.00E-178	526	632	3	*S. atrovirens*
A93	3.4	AY500257	Rhizobium sp. ORS 1439 16S ribosomal RNA gene, partial sequence	wild legume nodulating bacteria: Tunisia	90.87	416	4	6.00E-147	802	529	3	*S. atrovirens*
A93	1.5	DQ065033	Uncultured freshwater bacterium clone 965019A11.x1 16S ribosomal RNA gene, partial sequence	freshwater	89.91	545	22	0.0	747	673	3	*S. atrovirens*
A93	3.2	DQ154516	Uncultured soil bacterium clone RFS-C199 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	98.71	466	4	0.0	659	826	3	*S. atrovirens*
A93	2.1	DQ223206	Uncultured proteobacterium clone EV221H2111601SAH33 16S ribosomal RNA gene, partial sequence	subsurface water	94.66	524	18	0.0	727	804	3	*S. atrovirens*
A93	2.3	DQ223206	Uncultured proteobacterium clone EV221H2111601SAH33 16S ribosomal RNA gene, partial sequence	subsurface water	93.4	530	19	0.0	752	771	3	*S. atrovirens*
Blank	1	AF484679	Cloning vector pRV-9	replication competent; derived from RCAS(BP) avian retroviral vector	92.08	606	13	0.0	803	800	N/A	N/A
Blank	2	AJ585959	Thermococcales archaeon T30a-17 partial 16S rRNA gene	hydrothermal sample in a gas-lift bioreactor	99.52	414	0	0.0	543	752	N/A	N/A
BlankNC	3	AJ585959	Thermococcales archaeon T30a-17 partial 16S rRNA gene	hydrothermal sample in a gas-lift bioreactor	97.67	559	6	0.0	768	953	N/A	N/A
BlankNC	4	AJ585959	Thermococcales archaeon T30a-17 partial 16S rRNA gene	hydrothermal sample in a gas-lift bioreactor	89.72	574	9	0.0	736	691	N/A	N/A
SI5	2.1	AB113609	Uncultured beta proteobacterium gene for 16S rRNA, partial sequence, clone: HAuD-MB40	PCR-derived sequence from subsurface geothermal water	94.57	497	15	0.0	749	760	N/A	N/A
SI5	3.5	AF009989	Unidentified eubacterium 16S ribosomal RNA gene, partial sequence	soil	93.35	436	12	5.00E-177	707	628	N/A	N/A
SI5	3.4	AF424745	Uncultured bacterium clone M3rb1 16S ribosomal RNA gene, partial sequence	soil	92.81	445	13	5.00E-177	682	628	N/A	N/A
SI5	3.2	AF432823	Uncultured bacterium clone S39.44SM 16S ribosomal RNA gene	lodgepole pine rhizosphere soil	91.28	470	28	5.00E-177	773	628	N/A	N/A
SI5	1.3	AJ518795	Uncultured delta proteobacterium partial 16S rRNA gene, clone JG37-AG-90	uranium mining waste pile near Johanngeorgenstadt, soil sample	93.72	414	7	1.00E-169	1011	604	N/A	N/A
SI5	1.1	AY217493	Uncultured Acidobacteria bacterium clone WCB110 16S ribosomal RNA gene, partial sequence	saturated sediment, Wind Cave, South Dakota	91.72	507	14	0.0	692	682	N/A	N/A
SI5	1.2	AY360642	Uncultured Methylobacteriaceae bacterium clone M10Ba54 small subunit ribosomal RNA gene, partial sequence	oxic rice field soil	86.14	440	44	1.00E-128	653	468	N/A	N/A
SI5	1.4	AY921659	Uncultured Actinobacteria bacterium clone AKYG699 16S ribosomal RNA gene, partial sequence	farm soil adjacent to a silage storage bunker	87.18	390	18	6.00E-116	585	425	N/A	N/A
SI5	1.5	BX294823	Uncultured planctomycete partial 16S rRNA gene clone CY0ARA031A04 of environmental sample of uncultured planctomycete	aerobic basin from France	92.07	417	18	8.00E-160	670	571	N/A	N/A
SI5	3.3	DQ154408	Uncultured soil bacterium clone RFS-C81 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	84.09	484	21	2.00E-115	677	424	N/A	N/A
SI6	1.5	AB201600	Uncultured bacterium gene for 16S ribosomal RNA, partial sequence, clone: N-T-47	obtained from the experimental field at Nagano Chushin Agricultural Research Center, Japan	98.27	462	4	0.0	610	806	N/A	N/A
SI6	1.1	AJ232797	Unidentified eubacterium 16S rRNA gene (clone TRE2)	rhizoplane of Trifolium repens	92.28	324	17	4.00E-127	539	462	N/A	N/A
SI6	3.3	AJ863204	Uncultured bacterium partial 16S rRNA gene, clone 25BSU38	poplar tree microcosm, bulk soil, unflooded	89.72	399	20	2.00E-136	601	494	N/A	N/A

Sample	Plasmid Isolation ID	Accession #	Definition	Isolation Source	% Identity	Alignment Length	Mismatches	evalue	Sequence Length	Bit Score	Mesocosm Depth	Planting
SI6	3.1	AJ863223	Uncultured bacterium partial 16S rRNA gene, clone 25BSU19	poplar tree microcosm, bulk soil, unflooded	93.97	481	10	0.0	742	710	N/A	N/A
SI6	3.5	AJ876729	Uncultured bacterium partial 16S rRNA gene, clone R1_16	chromium contaminated wastewaters: river sediment	95.04	524	18	0.0	689	817	N/A	N/A
SI6	1.3	AY921896	Uncultured Acidobacteria bacterium clone AKYG1558 16S ribosomal RNA gene, partial sequence	farm soil adjacent to a silage storage bunker	91.91	408	18	5.00E-156	536	558	N/A	N/A
SI6	1.2	DQ066684	Uncultured bacterium clone FOTU12(1-6) 16S ribosomal RNA gene, partial sequence	bioreactor pretreating potable water	96.32	516	13	0.0	723	843	N/A	N/A
SI6	2.5	DQ154369	Uncultured soil bacterium clone RFS-C41 16S ribosomal RNA gene, partial sequence	Ross Forest Soil	95.12	471	7	0.0	762	732	N/A	N/A
SI6	1.4	DQ297986	Uncultured soil bacterium clone UC8 16S ribosomal RNA gene, partial sequence	hydrocarbon contaminated soil	92.28	531	17	0.0	781	734	N/A	N/A
SI6	3.4	DQ310755	Uncultured bacterium clone WCC77B-C01 16S ribosomal RNA gene, partial sequence	riverine sediment	92.6	527	27	0.0	749	749	N/A	N/A

Appendix O. RDP Classification

domain Bacteria (360 sequences)
»**phylum** Genera_incertae_sedis_OP11 (1)
» » » » » genus OP11 (1)
»**phylum** Genera_incertae_sedis_OP10 (1)
» » » » » genus OP10 (1)
»**phylum** Nitrospira (6 sequences)
» » class Nitrospira (6)
» » » order Nitrospirales (6)
» » » » family Nitrospiraceae (6)
» » » » » genus Nitrospira (6)
»**phylum** Verrucomicrobia (4 sequences)
» » class Verrucomicrobiae (4)
» » » order Verrucomicrobiales (4)
» » » » family Verrucomicrobiaceae (1)
» » » » » genus Verrucomicrobium (1)
» » » » unclassified Verrucomicrobiales (3)
»**phylum** Gemmatimonadetes (2 sequences)
» » class Gemmatimonadetes (2)
» » » order Gemmatimonadales (2)
» » » » family Gemmatimonadaceae (2)
» » » » » genus Gemmatimonas (2)
»**phylum** Bacteroidetes (11 sequences)
» » class Flavobacteria (1)
» » » order Flavobacteriales (1)
» » » » family Flavobacteriaceae (1)
» » » » » genus Flavobacterium (1)
» » class Bacteroidetes (1)
» » » order Bacteroidales (1)
» » » » family Porphyromonadaceae (1)
» » » » » unclassified Porphyromonadaceae (1)
» » class Sphingobacteria (2)
» » » order Sphingobacteriales (2)
» » » » family Crenotrichaceae (1)
» » » » » genus Chitinophaga (1)
» » » » family Flexibacteraceae (1)
» » » » » unclassified Flexibacteraceae (1)
» » unclassified Bacteroidetes (7)
»**phylum** Chloroflexi (12 sequences)
» » class Anaerolineae (12)
» » » order Anaerolinaeles (12)
» » » » family Anaerolinaeceea (12)
» » » » » genus Anaerolinea (12)
»**phylum** Planctomycetes (3 sequences)
» » class Planctomycetacia (3)
» » » order Planctomycetales (3)
» » » » family Planctomycetaceae (3)
» » » » » genus Planctomyces (1)
» » » » » unclassified Planctomycetaceae (2)
»**phylum** Actinobacteria (16 sequences)
» » class Actinobacteria (16)
» » » subclass Actinobacteridae (7)
» » » » order Actinomycetales (7)
» » » » » suborder Corynebacterineae (2)
» » » » » » family Mycobacteriaceae (1)
» » » » » » » genus Mycobacterium (1)
» » » » » unclassified Corynebacterineae (1)
» » » » » suborder Propionibacterineae (3)
» » » » » » family Nocardioidaceae (1)
» » » » » » » genus Nocardioides (1)
» » » » » » family Propionibacteriaceae (1)
» » » » » » » genus Microlunatus (1)
» » » » » » unclassified Propionibacterineae (1)
» » » » » unclassified Actinomycetales (2)
» » » subclass Rubrobacteridae (6)
» » » » order Rubrobacterales (6)
» » » » » suborder Rubrobacterineae (6)
» » » » » » family Rubrobacteraceae (6)
» » » » » » » genus Solirubrobacter (1)
» » » » » » » unclassified Rubrobacteraceae (5)
» » » unclassified Actinobacteria (3)
»**phylum** Proteobacteria (118 sequences)
» » class Gammaproteobacteria (9)
» » » order Xanthomonadales (3)
» » » » family Xanthomonadaceae (3)
» » » unclassified Gammaproteobacteria (6)
» » class Betaproteobacteria (42)
» » » order Rhodocyclales (1)
» » » » family Rhodocyclaceae (1)
» » » order Nitrosomonadales (1)
» » » » family Nitrosomonadaceae (1)
» » » order Burkholderiales (29)
» » » » family Incertae sedis 5 (1)
» » » » family Comamonadaceae (1)
» » » » unclassified Burkholderiales (27)
» » » unclassified Betaproteobacteria (11)
» » class Deltaproteobacteria (26)
» » » order Desulfobacterales (1)
» » » » family Desulfobacteraceae (1)
» » » order Desulfuromonales (5)
» » » » family Desulfuromonaceae (2)
» » » » family Geobacteraceae (1)
» » » » unclassified Desulfuromonales (2)
» » » order Myxococcales (5)
» » » » suborder Nannocystineae (1)
» » » » suborder Cystobacterineae (3)
» » » » unclassified Myxococcales (1)
» » » unclassified Deltaproteobacteria (15)
» » class Alphaproteobacteria (20)
» » » order Caulobacterales (1)
» » » » family Caulobacteraceae (1)
» » » order Sphingomonadales (1)
» » » » family Sphingomonadaceae (1)
» » » order Rhizobiales (11)
» » » » family Hyphomicrobiaceae (2)
» » » » family Phyllobacteriaceae (2)
» » » » family Bradyrhizobiaceae (1)
» » » » unclassified Rhizobiales (6)
» » » order Rhodospirillales (1)
» » » » unclassified Rhodospirillales (1)
» » » unclassified Alphaproteobacteria (6)
» » unclassified Proteobacteria (21)
»**phylum** Firmicutes (3 sequences)
» » class Bacilli (1)
» » » order Bacillales (1)
» » » » family Bacillaceae (1)
» » » » » unclassified Bacillaceae (1)
» » class Clostridia (2)
» » » order Clostridiales (1)
» » » » family Clostridiaceae (1)
» » » » » genus Acetivibrio (1)
» » » unclassified Clostridia (1)
» **unclassified Bacteria (183)**
unclassified Phylum(2)

Classifier: Naive Bayesian rRNA Classifier Version 1.0, November 2003

Taxonomical Hierarchy: Bergey's Manual of Systematic Bacteriology, release 6.0

Submit Date: Mon Apr 17 07:34:40 EDT 2006

Confidence threshold: 80%

Symbol - after a sequence name indicates the results are obtained using reverse complement of that query sequence.

Lineage: Root(362)

Details:

Mesocosm Depth	Sample	Domain		Phylum		Class		Subclass		Order		Suborder		Family		Genus	
3	A73.2.3	Bacteria	100%	Firmicutes	100%	Clostridia	100%			Clostridiales	100%			Clostridiaceae	100%	Acetivibrio	100%
3	A63.3.2	Bacteria	100%	Firmicutes	47%	Clostridia	46%			Clostridiales	29%			Acidaminococcaceae	17%	Acetonema	7%
1	A31.2.1.2	Bacteria	93%	Proteobacteria	36%	Gammaproteobacteria	13%			Thiotrichales	7%			Thiotrichaceae	7%	Achromatium	7%
2	A22.2.2.3	Bacteria	100%	Actinobacteria	96%	Actinobacteria	96%	Acidimicrobidae	48%	Acidimicrobiales	48%	Acidimicrobineae	48%	Acidimicrobiaceae	48%	Acidimicrobium	48%
3	A10-3.2.4	Bacteria	100%	Acidobacteria	38%	Acidobacteria	38%			Acidobacteriales	38%			Acidobacteriaceae	38%	Acidobacterium	35%
1	A11-1.3.5	Bacteria	100%	Acidobacteria	51%	Acidobacteria	51%			Acidobacteriales	51%			Acidobacteriaceae	51%	Acidobacterium	51%
2	A12.3.2	Bacteria	90%	Acidobacteria	35%	Acidobacteria	35%			Acidobacteriales	35%			Acidobacteriaceae	35%	Acidobacterium	34%
2	A32.2.2.1	Bacteria	100%	Acidobacteria	65%	Acidobacteria	65%			Acidobacteriales	65%			Acidobacteriaceae	65%	Acidobacterium	58%
1	A51.3.6	Bacteria	100%	Acidobacteria	62%	Acidobacteria	62%			Acidobacteriales	62%			Acidobacteriaceae	62%	Acidobacterium	60%
2	A52.3.2	Bacteria	99%	Acidobacteria	33%	Acidobacteria	33%			Acidobacteriales	33%			Acidobacteriaceae	33%	Acidobacterium	25%
2	A62.2.3	Bacteria	100%	Acidobacteria	51%	Acidobacteria	51%			Acidobacteriales	51%			Acidobacteriaceae	51%	Acidobacterium	27%
3	A63.2.2	Bacteria	100%	Acidobacteria	77%	Acidobacteria	77%			Acidobacteriales	77%			Acidobacteriaceae	77%	Acidobacterium	76%
3	A73.1.3	Bacteria	99%	Acidobacteria	37%	Acidobacteria	37%			Acidobacteriales	37%			Acidobacteriaceae	37%	Acidobacterium	29%
3	A83.2.5	Bacteria	100%	Acidobacteria	40%	Acidobacteria	40%			Acidobacteriales	40%			Acidobacteriaceae	40%	Acidobacterium	29%
3	A10-3.2.1	Bacteria	99%	Actinobacteria	94%	Actinobacteria	94%	Actinobacteridae	75%	Actinomycetales	75%	Frankineae	57%	Acidothermaceae	41%	Acidothermus	41%
2	A22.2.3.1	Bacteria	100%	Actinobacteria	88%	Actinobacteria	88%	Actinobacteridae	57%	Actinomycetales	57%	Frankineae	39%	Acidothermaceae	31%	Acidothermus	31%
1	A71.1.1	Bacteria	97%	Actinobacteria	72%	Actinobacteria	72%	Actinobacteridae	42%	Actinomycetales	42%	Frankineae	25%	Acidothermaceae	13%	Acidothermus	13%
3	A13.3.1	Bacteria	100%	Proteobacteria	100%	Alphaproteobacteria	100%			Rhizobiales	100%			Bradyrhizobiaceae	100%	Afipia	68%
2	A72.2.4	Bacteria	100%	Proteobacteria	100%	Alphaproteobacteria	100%			Rhizobiales	94%			Bradyrhizobiaceae	43%	Afipia	18%
N/A	SI5.3.5	Bacteria	91%	Proteobacteria	37%	Alphaproteobacteria	11%			Rhizobiales	6%			Bradyrhizobiaceae	5%	Agromonas	5%
1	A41.1.8	Bacteria	100%	Firmicutes	34%	Clostridia	32%			Clostridiales	25%			Acidaminococcaceae	21%	Allisonella	7%
2	A72.1.4	Bacteria	97%	Firmicutes	37%	Clostridia	34%			Clostridiales	28%			Acidaminococcaceae	14%	Allisonella	5%
1	A10-1.2.1	Bacteria	68%	Proteobacteria	17%	Gammaproteobacteria	8%			Enterobacteriales	3%			Enterobacteriaceae	3%	Alterococcus	3%
1	A10-1.2.6	Bacteria	99%	Proteobacteria	40%	Gammaproteobacteria	18%			Enterobacteriales	11%			Enterobacteriaceae	11%	Alterococcus	9%
3	A33.2.1.4	Bacteria	100%	Proteobacteria	23%	Gammaproteobacteria	13%			Enterobacteriales	6%			Enterobacteriaceae	6%	Alterococcus	6%
1	A81.1.5	Bacteria	100%	Proteobacteria	66%	Gammaproteobacteria	47%			Enterobacteriales	47%			Enterobacteriaceae	47%	Alterococcus	47%
2	A72.1.1	Bacteria	100%	Firmicutes	33%	Clostridia	32%			Clostridiales	23%			Acidaminococcaceae	16%	Anaeroarcus	7%
2	A10-2.2.3	Bacteria	100%	Chloroflexi	90%	Anaerolineae	90%			Anaerolinaeles	90%			Anaerolinaeceea	90%	Anaerolinea	90%
3	A11-3.1.2	Bacteria	100%	Chloroflexi	100%	Anaerolineae	100%			Anaerolinaeles	100%			Anaerolinaeceea	100%	Anaerolinea	100%
3	A11-3.3.1	Bacteria	100%	Chloroflexi	39%	Anaerolineae	33%			Anaerolinaeles	33%			Anaerolinaeceea	33%	Anaerolinea	33%
3	A11-3.3.5	Bacteria	100%	Chloroflexi	100%	Anaerolineae	100%			Anaerolinaeles	100%			Anaerolinaeceea	100%	Anaerolinea	100%
1	A12-1.3.4	Bacteria	100%	Chloroflexi	100%	Anaerolineae	100%			Anaerolinaeles	100%			Anaerolinaeceea	100%	Anaerolinea	100%
1	A21.2.3.1	Bacteria	100%	Chloroflexi	99%	Anaerolineae	99%			Anaerolinaeles	99%			Anaerolinaeceea	99%	Anaerolinea	99%
2	A32.2.2.4	Bacteria	99%	Chloroflexi	20%	Anaerolineae	20%			Anaerolinaeles	20%			Anaerolinaeceea	20%	Anaerolinea	20%
1	A51.1.1	Bacteria	100%	Chloroflexi	100%	Anaerolineae	100%			Anaerolinaeles	100%			Anaerolinaeceea	100%	Anaerolinea	100%
1	A51.2.5	Bacteria	100%	Chloroflexi	99%	Anaerolineae	99%			Anaerolinaeles	99%			Anaerolinaeceea	99%	Anaerolinea	99%
2	A52.2.2	Bacteria	100%	Chloroflexi	91%	Anaerolineae	91%			Anaerolinaeles	91%			Anaerolinaeceea	91%	Anaerolinea	91%
2	A52.3.3	Bacteria	100%	Chloroflexi	97%	Anaerolineae	97%			Anaerolinaeles	97%			Anaerolinaeceea	97%	Anaerolinea	97%
3	A53.3.4	Bacteria	100%	Chloroflexi	100%	Anaerolineae	100%			Anaerolinaeles	100%			Anaerolinaeceea	100%	Anaerolinea	100%
1	A71.1.5	Bacteria	99%	Chloroflexi	55%	Anaerolineae	55%			Anaerolinaeles	55%			Anaerolinaeceea	55%	Anaerolinea	55%
1	A81.1.2	Bacteria	100%	Chloroflexi	100%	Anaerolineae	100%			Anaerolinaeles	100%			Anaerolinaeceea	100%	Anaerolinea	100%
1	A81.3.4	Bacteria	100%	Chloroflexi	99%	Anaerolineae	99%			Anaerolinaeles	99%			Anaerolinaeceea	99%	Anaerolinea	99%
N/A	SI5.1.1	Bacteria	90%	Firmicutes	39%	Clostridia	38%			Clostridiales	26%			Acidaminococcaceae	10%	Anaeromusa	5%
1	A10-1.2.3	Bacteria	99%	Proteobacteria	68%	Deltaproteobacteria	59%			Myxococcales	23%	Cystobacterineae	17%	Cystobacteraceae	16%	Anaeromyxobacter	7%
1	A12-1.1.1	Bacteria	100%	Proteobacteria	57%	Deltaproteobacteria	57%			Myxococcales	42%	Cystobacterineae	42%	Cystobacteraceae	42%	Anaeromyxobacter	41%
2	A12-2.3.3	Bacteria	98%	Proteobacteria	87%	Deltaproteobacteria	63%			Myxococcales	55%	Cystobacterineae	53%	Cystobacteraceae	52%	Anaeromyxobacter	51%

Mesocosm Depth	Sample	Domain		Phylum		Class		Subclass		Order		Suborder		Family		Genus	
3	A12-3.1.3	Bacteria	100%	Proteobacteria	100%	Deltaproteobacteria	100%			Myxococcales	100%	Cystobacterineae	100%	Cystobacteraceae	100%	Anaeromyxobacter	100%
3	A12-3.3.4	Bacteria	100%	Proteobacteria	89%	Deltaproteobacteria	88%			Myxococcales	38%	Cystobacterineae	30%	Cystobacteraceae	30%	Anaeromyxobacter	24%
3	A23.2.1.3	Bacteria	100%	Proteobacteria	100%	Deltaproteobacteria	100%			Myxococcales	100%	Cystobacterineae	100%	Cystobacteraceae	100%	Anaeromyxobacter	100%
2	A62.2.4	Bacteria	100%	Proteobacteria	84%	Deltaproteobacteria	83%			Myxococcales	52%	Cystobacterineae	51%	Cystobacteraceae	49%	Anaeromyxobacter	34%
N/A	SI5.1.3	Bacteria	92%	Proteobacteria	60%	Deltaproteobacteria	54%			Myxococcales	24%	Cystobacterineae	21%	Cystobacteraceae	21%	Anaeromyxobacter	19%
1	A10-1.2.5	Bacteria	99%	Bacteroidetes	83%	Bacteroidetes	38%			Bacteroidales	38%			Bacteroidaceae	26%	Anaerophaga	26%
1	A11.2.3.5	Bacteria	100%	Bacteroidetes	100%	Bacteroidetes	72%			Bacteroidales	72%			Bacteroidaceae	37%	Anaerophaga	37%
3	A12-3.3.3	Bacteria	100%	Bacteroidetes	86%	Bacteroidetes	62%			Bacteroidales	62%			Bacteroidaceae	33%	Anaerophaga	33%
1	A61.2.5	Bacteria	100%	Bacteroidetes	98%	Bacteroidetes	59%			Bacteroidales	59%			Bacteroidaceae	27%	Anaerophaga	27%
2	A42.2.6	Bacteria	95%	Firmicutes	40%	Clostridia	28%			Clostridiales	27%			Acidaminococcaceae	12%	Anaerosinus	3%
2	A62.3.5	Bacteria	100%	Firmicutes	38%	Clostridia	38%			Clostridiales	26%			Acidaminococcaceae	16%	Anaerovibrio	6%
2	A11-2.3.2	Bacteria	99%	Firmicutes	28%	Clostridia	24%			Clostridiales	19%			Clostridiaceae	18%	Anoxynatronum	12%
1	A41.3.6	Bacteria	97%	Aquificae	33%	Aquificae	33%			Aquificales	33%			Aquificaceae	21%	Aquifex	10%
1	A81.3.5	Bacteria	96%	Aquificae	16%	Aquificae	16%			Aquificales	16%			Aquificaceae	16%	Aquifex	10%
2	A92.2.1	Bacteria	99%	Aquificae	20%	Aquificae	20%			Aquificales	20%			Aquificaceae	20%	Aquifex	20%
1	A61.1.3	Bacteria	99%	Proteobacteria	26%	Deltaproteobacteria	10%			Myxococcales	7%	Cystobacterineae	7%	Cystobacteraceae	5%	Archangium	5%
1	A71.2.2	Bacteria	100%	Proteobacteria	94%	Deltaproteobacteria	55%			Myxococcales	34%	Cystobacterineae	34%	Cystobacteraceae	22%	Archangium	16%
N/A	SI5.3.4	Bacteria	100%	Bacteroidetes	97%	Sphingobacteria	87%			Sphingobacteriales	87%			Flexibacteraceae	86%	Arcicella	29%
2	A32.2.3.4	Bacteria	100%	Actinobacteria	99%	Actinobacteria	99%	Actinobacteridae	93%	Actinomycetales	93%	Micrococcineae	24%	Intrasporangiaceae	22%	Arsenicicoccus	16%
2	A82.1.5	Bacteria	100%	Firmicutes	100%	Bacilli	100%			Bacillales	100%			Bacillaceae	100%	Bacillus	77%
2	A12-2.1.1	Bacteria	100%	Proteobacteria	34%	Alphaproteobacteria	27%			Rhizobiales	23%			Bradyrhizobiaceae	22%	Balneimonas	21%
2	A72.2.2	Bacteria	99%	Proteobacteria	85%	Deltaproteobacteria	75%			Bdellovibrionales	20%			Bdellovibrionaceae	19%	Bdellovibrio	19%
3	A93.3.4	Bacteria	100%	Proteobacteria	100%	Alphaproteobacteria	100%			Rhizobiales	97%			Bradyrhizobiaceae	37%	Blastobacter	37%
3	A43.2.2.5	Bacteria	100%	Planctomycetes	23%	Planctomycetacia	23%			Planctomycetales	23%			Planctomycetaceae	23%	Blastopirellula	23%
N/A	SI5.1.5	Bacteria	99%	Planctomycetes	97%	Planctomycetacia	97%			Planctomycetales	97%			Planctomycetaceae	97%	Blastopirellula	50%
1	A41.3.8	Bacteria	100%	Proteobacteria	96%	Alphaproteobacteria	93%			Rhizobiales	79%			Bradyrhizobiaceae	32%	Bosea	13%
2	A92.2.4	Bacteria	100%	Proteobacteria	100%	Alphaproteobacteria	100%			Caulobacterales	85%			Caulobacteraceae	85%	Brevundimonas	76%
3	A10-3.2.2	Bacteria	100%	Proteobacteria	98%	Betaproteobacteria	98%			Burkholderiales	97%			Comamonadaceae	39%	Caenibacterium	24%
1	A12-1.2.4	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	98%			Burkholderiales	95%			Comamonadaceae	49%	Caenibacterium	25%
1	A12-1.3.1	Bacteria	100%	Proteobacteria	61%	Betaproteobacteria	37%			Burkholderiales	36%			Comamonadaceae	33%	Caenibacterium	31%
2	A12-2.3.1	Bacteria	100%	Proteobacteria	95%	Betaproteobacteria	92%			Burkholderiales	85%			Comamonadaceae	60%	Caenibacterium	48%
2	A22.2.3.4	Bacteria	100%	Proteobacteria	93%	Betaproteobacteria	87%			Burkholderiales	62%			Comamonadaceae	37%	Caenibacterium	32%
3	A33.2.3.2	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	100%			Burkholderiales	97%			Comamonadaceae	43%	Caenibacterium	37%
1	A41.3.1	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	100%			Burkholderiales	98%			Comamonadaceae	62%	Caenibacterium	54%
3	A43.2.3.2	Bacteria	100%	Proteobacteria	95%	Betaproteobacteria	88%			Burkholderiales	85%			Comamonadaceae	45%	Caenibacterium	37%
2	A82.2.4	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	98%			Burkholderiales	88%			Comamonadaceae	55%	Caenibacterium	52%
3	A93.2.5	Bacteria	100%	Proteobacteria	99%	Betaproteobacteria	97%			Burkholderiales	86%			Comamonadaceae	58%	Caenibacterium	56%
N/A	SI6.3.1	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	96%			Burkholderiales	92%			Comamonadaceae	62%	Caenibacterium	60%
1	A10-1.2.4	Bacteria	95%	Proteobacteria	28%	Betaproteobacteria	12%			Burkholderiales	6%			Comamonadaceae	3%	Caldimonas	3%
1	A41.2.4	Bacteria	57%	Firmicutes	26%	Clostridia	26%			Clostridiales	17%			Lachnospiraceae	7%	Catenibacterium	6%
3	A73.3.4	Bacteria	100%	Firmicutes	37%	Clostridia	36%			Clostridiales	26%			Lachnospiraceae	9%	Catenibacterium	8%
3	A23.2.1.4	Bacteria	97%	Firmicutes	41%	Clostridia	32%			Clostridiales	26%			Acidaminococcaceae	7%	Centipeda	2%
1	A21.2.2.1	Bacteria	100%	Proteobacteria	36%	Betaproteobacteria	12%			Neisseriales	7%			Neisseriaceae	7%	Chitinibacter	5%
3	A83.1.3	Bacteria	98%	Proteobacteria	47%	Betaproteobacteria	23%			Burkholderiales	11%			Burkholderiaceae	9%	Chitinimonas	8%
2	A12-2.1.5	Bacteria	100%	Bacteroidetes	100%	Sphingobacteria	100%			Sphingobacteriales	100%			Crenotrichaceae	84%	Chitinophaga	84%
2	A72.2.5	Bacteria	100%	Proteobacteria	100%	Deltaproteobacteria	100%			Myxococcales	97%	Sorangineae	70%	Polyangiaceae	70%	Chondromyces	70%
3	A53.2.2	Bacteria	100%	Proteobacteria	97%	Gammaproteobacteria	62%			Chromatiales	24%			Chromatiaceae	22%	Chromatium	20%
2	A62.3.1	Bacteria	100%	Bacteroidetes	100%	Flavobacteria	45%			Flavobacteriales	45%			Flavobacteriaceae	44%	Coenonia	19%
2	A92.1.2	Bacteria	100%	Proteobacteria	34%	Betaproteobacteria	10%			Burkholderiales	6%			Oxalobacteraceae	5%	Collimonas	2%
2	A10-2.2.4	Bacteria	100%	Actinobacteria	64%	Actinobacteria	64%	Rubrobacteridae	50%	Rubrobacterales	50%	Rubrobacterineae	50%	Rubrobacteraceae	50%	Conexibacter	27%
1	A11-1.1.1	Bacteria	100%	Actinobacteria	59%	Actinobacteria	59%	Rubrobacteridae	41%	Rubrobacterales	41%	Rubrobacterineae	41%	Rubrobacteraceae	41%	Conexibacter	14%
2	A11-2.2.3	Bacteria	100%	Actinobacteria	63%	Actinobacteria	63%	Rubrobacteridae	54%	Rubrobacterales	54%	Rubrobacterineae	54%	Rubrobacteraceae	54%	Conexibacter	26%
3	A33.2.1.2	Bacteria	100%	Actinobacteria	100%	Actinobacteria	100%	Rubrobacteridae	100%	Rubrobacterales	100%	Rubrobacterineae	100%	Rubrobacteraceae	100%	Conexibacter	65%

Mesocosm Depth	Sample	Domain		Phylum		Class		Subclass		Order		Suborder		Family		Genus	
3	A43.2.3.5	Bacteria	100%	Actinobacteria	57%	Actinobacteria	57%	Rubrobacteridae	33%	Rubrobacterales	33%	Rubrobacterineae	33%	Rubrobacteraceae	33%	Conexibacter	31%
1	A71.3.4	Bacteria	99%	Actinobacteria	44%	Actinobacteria	44%	Rubrobacteridae	33%	Rubrobacterales	33%	Rubrobacterineae	33%	Rubrobacteraceae	33%	Conexibacter	22%
3	A93.3.5	Bacteria	100%	Actinobacteria	97%	Actinobacteria	97%	Rubrobacteridae	96%	Rubrobacterales	96%	Rubrobacterineae	96%	Rubrobacteraceae	96%	Conexibacter	59%
N/A	SI5.1.4	Bacteria	99%	Actinobacteria	85%	Actinobacteria	85%	Rubrobacteridae	85%	Rubrobacterales	85%	Rubrobacterineae	85%	Rubrobacteraceae	85%	Conexibacter	78%
1	A11.2.2.1	Bacteria	88%	Proteobacteria	46%	Alphaproteobacteria	18%			Rhodospirillales	7%			Acetobacteraceae	7%	Craurococcus	3%
2	A11-2.1.1	Bacteria	100%	Proteobacteria	28%	Alphaproteobacteria	12%			Rhodospirillales	11%			Acetobacteraceae	9%	Craurococcus	7%
2	A11-2.2.5	Bacteria	100%	Proteobacteria	61%	Alphaproteobacteria	49%			Rhodospirillales	47%			Acetobacteraceae	31%	Craurococcus	16%
2	A12-2.2.1	Bacteria	99%	Proteobacteria	64%	Alphaproteobacteria	33%			Rhodospirillales	32%			Acetobacteraceae	30%	Craurococcus	13%
2	A82.1.4	Bacteria	93%	Proteobacteria	42%	Alphaproteobacteria	38%			Rhodospirillales	37%			Acetobacteraceae	35%	Craurococcus	10%
1	A12-1.1.2	Bacteria	100%	Firmicutes	34%	Clostridia	32%			Clostridiales	22%			Peptococcaceae	9%	Cryptanaerobacter	9%
3	A73.2.5	Bacteria	100%	Firmicutes	51%	Clostridia	49%			Clostridiales	41%			Peptococcaceae	16%	Cryptanaerobacter	14%
2	A42.1.8	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	100%			Burkholderiales	97%			Burkholderiaceae	59%	Cupriavidus	58%
3	A83.3.3	Bacteria	98%	Proteobacteria	83%	Betaproteobacteria	74%			Burkholderiales	69%			Burkholderiaceae	38%	Cupriavidus	20%
1	A10-1.1.3	Bacteria	100%	Proteobacteria	57%	Gammaproteobacteria	20%			Thiotrichales	16%			Piscirickettsiaceae	12%	Cycloclasticus	12%
3	A12-3.1.1	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	100%			Rhodocyclales	94%			Rhodocyclaceae	94%	Dechlorosoma	61%
2	A52.2.3	Bacteria	99%	Firmicutes	40%	Clostridia	38%			Clostridiales	26%			Peptococcaceae	9%	Dehalobacter	5%
3	A43.2.1.2	Bacteria	99%	Proteobacteria	79%	Deltaproteobacteria	72%			Syntrophobacterales	34%			Syntrophaceae	24%	Desulfobacca	21%
3	A83.2.2	Bacteria	100%	Proteobacteria	92%	Deltaproteobacteria	89%			Syntrophobacterales	51%			Syntrophaceae	32%	Desulfobacca	26%
1	A51.2.2	Bacteria	99%	Proteobacteria	57%	Deltaproteobacteria	31%			Desulfobacterales	12%			Desulfobacteraceae	12%	Desulfobotulus	11%
1	A41.2.7	Bacteria	99%	Proteobacteria	53%	Deltaproteobacteria	40%			Desulfobacterales	30%			Desulfobacteraceae	29%	Desulfocella	27%
1	A41.3.7	Bacteria	100%	Proteobacteria	52%	Deltaproteobacteria	25%			Desulfobacterales	19%			Desulfobacteraceae	19%	Desulfocella	16%
1	A10-1.2.7	Bacteria	100%	Proteobacteria	94%	Deltaproteobacteria	91%			Desulfovibrionales	36%			Desulfohalobiaceae	21%	Desulfonatronovibrio	15%
1	A11-1.3.2	Bacteria	100%	Proteobacteria	53%	Deltaproteobacteria	22%			Desulfovibrionales	9%			Desulfohalobiaceae	8%	Desulfonatronovibrio	8%
3	A83.1.2	Bacteria	100%	Proteobacteria	36%	Deltaproteobacteria	28%			Desulfovibrionales	25%			Desulfohalobiaceae	18%	Desulfonauticus	18%
1	A91.1.5	Bacteria	99%	Proteobacteria	32%	Deltaproteobacteria	10%			Desulfovibrionales	4%			Desulfohalobiaceae	4%	Desulfonauticus	3%
3	A10-3.3.4	Bacteria	98%	Proteobacteria	72%	Deltaproteobacteria	55%			Desulfobacterales	45%			Desulfobacteraceae	45%	Desulforegula	14%
1	A12-1.2.3	Bacteria	100%	Proteobacteria	100%	Deltaproteobacteria	100%			Desulfobacterales	99%			Desulfobacteraceae	99%	Desulforegula	99%
3	A13.2.5	Bacteria	100%	Proteobacteria	56%	Deltaproteobacteria	45%			Desulfobacterales	18%			Desulfobacteraceae	15%	Desulforegula	14%
2	A42.3.7	Bacteria	100%	Proteobacteria	52%	Deltaproteobacteria	31%			Desulfobacterales	22%			Desulfobacteraceae	22%	Desulforegula	9%
3	A43.2.3.4	Bacteria	100%	Proteobacteria	63%	Deltaproteobacteria	21%			Desulfobacterales	16%			Desulfobacteraceae	16%	Desulforegula	15%
1	A81.1.4	Bacteria	100%	Proteobacteria	62%	Deltaproteobacteria	56%			Desulfobacterales	28%			Desulfobacteraceae	28%	Desulforegula	14%
2	A82.1.1	Bacteria	99%	Proteobacteria	82%	Deltaproteobacteria	75%			Desulfobacterales	30%			Desulfobacteraceae	29%	Desulforegula	28%
3	A10-3.3.3	Bacteria	96%	Proteobacteria	29%	Deltaproteobacteria	13%			Syntrophobacterales	12%			Syntrophobacteraceae	12%	Desulforhabdus	8%
2	A11-2.1.2	Bacteria	100%	Proteobacteria	82%	Deltaproteobacteria	81%			Syntrophobacterales	43%			Syntrophobacteraceae	26%	Desulforhabdus	16%
1	A41.1.6	Bacteria	100%	Proteobacteria	92%	Deltaproteobacteria	92%			Syntrophobacterales	42%			Syntrophobacteraceae	40%	Desulforhabdus	31%
N/A	SI6.1.5	Bacteria	98%	Proteobacteria	53%	Deltaproteobacteria	26%			Syntrophobacterales	9%			Syntrophobacteraceae	8%	Desulforhabdus	7%
3	A63.2.4	Bacteria	100%	Proteobacteria	95%	Deltaproteobacteria	71%			Desulfobacterales	38%			Desulfobacteraceae	37%	Desulfospira	20%
1	A10-1.1.1	Bacteria	100%	Proteobacteria	65%	Deltaproteobacteria	60%			Syntrophobacterales	28%			Syntrophobacteraceae	28%	Desulfovirga	27%
1	A41.1.7	Bacteria	99%	Proteobacteria	43%	Deltaproteobacteria	32%			Syntrophobacterales	19%			Syntrophobacteraceae	16%	Desulfovirga	12%
1	A51.3.8	Bacteria	100%	Proteobacteria	49%	Deltaproteobacteria	31%			Syntrophobacterales	10%			Syntrophobacteraceae	5%	Desulfovirga	4%
3	A83.2.4	Bacteria	99%	Proteobacteria	80%	Deltaproteobacteria	73%			Syntrophobacterales	19%			Syntrophobacteraceae	12%	Desulfovirga	12%
1	A11.2.3.3	Bacteria	100%	Bacteroidetes	98%	Bacteroidetes	84%			Bacteroidales	84%			Porphyromonadaceae	81%	Dysgonomonas	64%
1	A11-1.3.3	Bacteria	100%	Bacteroidetes	78%	Bacteroidetes	44%			Bacteroidales	44%			Porphyromonadaceae	33%	Dysgonomonas	30%
1	A71.3.2	Bacteria	100%	Actinobacteria	77%	Actinobacteria	77%	Coriobacteridae	64%	Coriobacteriales	64%	Coriobacterineae	64%	Coriobacteriaceae	64%	Eggerthella	42%
1	A41.1.5	Bacteria	98%	Proteobacteria	72%	Deltaproteobacteria	36%			Myxococcales	22%	Nannocystineae	14%	Nannocystaceae	14%	Enhygromyxa	12%
1	A10-1.3.4	Bacteria	100%	Firmicutes	56%	Clostridia	55%			Clostridiales	44%			Clostridiaceae	22%	Faecalibacterium	13%
2	A11-2.3.1	Bacteria	100%	Firmicutes	57%	Clostridia	56%			Clostridiales	50%			Clostridiaceae	23%	Faecalibacterium	20%
1	A21.2.2.5	Bacteria	100%	Firmicutes	54%	Clostridia	54%			Clostridiales	45%			Clostridiaceae	27%	Faecalibacterium	22%
3	A23.2.2	Bacteria	100%	Firmicutes	31%	Clostridia	31%			Clostridiales	27%			Clostridiaceae	16%	Faecalibacterium	14%
2	A32.2.2.3	Bacteria	100%	Firmicutes	48%	Clostridia	47%			Clostridiales	42%			Clostridiaceae	28%	Faecalibacterium	27%
3	A53.3.3	Bacteria	100%	Firmicutes	46%	Clostridia	44%			Clostridiales	35%			Clostridiaceae	25%	Faecalibacterium	21%
2	A62.1.5	Bacteria	100%	Firmicutes	38%	Clostridia	36%			Clostridiales	32%			Clostridiaceae	20%	Faecalibacterium	17%
3	A73.2.4	Bacteria	100%	Firmicutes	47%	Clostridia	46%			Clostridiales	39%			Clostridiaceae	27%	Faecalibacterium	21%

Mesocosm Depth	Sample	Domain		Phylum		Class		Subclass		Order		Suborder		Family		Genus	
1	A81.1.1	Bacteria	100%	Firmicutes	66%	Clostridia	64%			Clostridiales	60%			Clostridiaceae	35%	Faecalibacterium	30%
1	A81.1.3	Bacteria	100%	Firmicutes	42%	Clostridia	41%			Clostridiales	40%			Clostridiaceae	16%	Faecalibacterium	13%
2	A82.3.5	Bacteria	100%	Firmicutes	39%	Clostridia	39%			Clostridiales	39%			Clostridiaceae	24%	Faecalibacterium	24%
1	A91.2.1	Bacteria	99%	Firmicutes	35%	Clostridia	32%			Clostridiales	30%			Clostridiaceae	15%	Faecalibacterium	6%
3	A53.2.1	Bacteria	100%	Proteobacteria	100%	Alphaproteobacteria	87%			Rhizobiales	52%			Hyphomicrobiaceae	45%	Filomicrobium	40%
2	A62.1.2	Bacteria	100%	Proteobacteria	95%	Gammaproteobacteria	88%			Pseudomonadales	72%			Pseudomonadaceae	61%	Flavimonas	49%
2	A32.2.1.4	Bacteria	100%	Bacteroidetes	100%	Flavobacteria	100%			Flavobacteriales	100%			Flavobacteriaceae	100%	Flavobacterium	100%
3	A13.2.7	Bacteria	100%	Proteobacteria	45%	Gammaproteobacteria	17%			Xanthomonadales	7%			Xanthomonadaceae	7%	Fulvimonas	5%
2	A12-2.1.2	Bacteria	86%	Firmicutes	31%	Clostridia	19%			Thermoanaerobacteriales	9%			Thermoanaerobacteriacea	8%	Gelria	8%
3	A33.2.2.1	Bacteria	100%	Planctomycetes	87%	Planctomycetacia	87%			Planctomycetales	87%			Planctomycetaceae	87%	Gemmata	70%
3	A10-3.1.5	Bacteria	100%	Gemmatimonadetes	83%	Gemmatimonadetes	83%			Gemmatimonadales	83%			Gemmatimonadaceae	83%	Gemmatimonas	83%
3	A10-3.2.3	Bacteria	100%	Gemmatimonadetes	76%	Gemmatimonadetes	76%			Gemmatimonadales	76%			Gemmatimonadaceae	76%	Gemmatimonas	76%
1	A12-1.3.2	Bacteria	100%	Gemmatimonadetes	68%	Gemmatimonadetes	68%			Gemmatimonadales	68%			Gemmatimonadaceae	68%	Gemmatimonas	68%
3	A13.2.6	Bacteria	100%	Gemmatimonadetes	75%	Gemmatimonadetes	75%			Gemmatimonadales	75%			Gemmatimonadaceae	75%	Gemmatimonas	75%
3	A13.3.4	Bacteria	100%	Gemmatimonadetes	80%	Gemmatimonadetes	80%			Gemmatimonadales	80%			Gemmatimonadaceae	80%	Gemmatimonas	80%
1	A61.1.4	Bacteria	100%	Gemmatimonadetes	50%	Gemmatimonadetes	50%			Gemmatimonadales	50%			Gemmatimonadaceae	50%	Gemmatimonas	50%
1	A61.3.5	Bacteria	98%	Gemmatimonadetes	63%	Gemmatimonadetes	63%			Gemmatimonadales	63%			Gemmatimonadaceae	63%	Gemmatimonas	63%
1	A71.2.3	Bacteria	100%	Gemmatimonadetes	68%	Gemmatimonadetes	68%			Gemmatimonadales	68%			Gemmatimonadaceae	68%	Gemmatimonas	68%
3	A73.1.1	Bacteria	97%	Gemmatimonadetes	59%	Gemmatimonadetes	59%			Gemmatimonadales	59%			Gemmatimonadaceae	59%	Gemmatimonas	59%
1	A81.2.2	Bacteria	100%	Gemmatimonadetes	73%	Gemmatimonadetes	73%			Gemmatimonadales	73%			Gemmatimonadaceae	73%	Gemmatimonas	73%
N/A	SI6.1.4	Bacteria	99%	Gemmatimonadetes	41%	Gemmatimonadetes	41%			Gemmatimonadales	41%			Gemmatimonadaceae	41%	Gemmatimonas	41%
2	A12.3.1	Bacteria	100%	Proteobacteria	100%	Deltaproteobacteria	100%			Desulfuromonales	100%			Geobacteraceae	51%	Geobacter	46%
2	A42.2.3	Bacteria	100%	Proteobacteria	100%	Deltaproteobacteria	100%			Desulfuromonales	100%			Geobacteraceae	96%	Geobacter	91%
3	A73.3.3	Bacteria	100%	Proteobacteria	100%	Deltaproteobacteria	99%			Desulfuromonales	99%			Geobacteraceae	78%	Geobacter	70%
1	A11.2.1.1	Bacteria	100%	Proteobacteria	98%	Deltaproteobacteria	90%			Myxococcales	90%	Nannocystineae	88%	Haliangiaceae	87%	Haliangium	87%
3	A10-3.3.5	Bacteria	98%	Bacteroidetes	67%	Sphingobacteria	60%			Sphingobacteriales	60%			Saprospiraceae	52%	Haliscomenobacter	38%
1	A61.1.1	Bacteria	93%	Bacteroidetes	29%	Sphingobacteria	28%			Sphingobacteriales	28%			Saprospiraceae	25%	Haliscomenobacter	20%
3	A53.1.5	Bacteria	100%	Proteobacteria	99%	Gammaproteobacteria	75%			Chromatiales	50%			Chromatiaceae	46%	Halochromatium	37%
1	A71.3.1	Bacteria	100%	Proteobacteria	56%	Gammaproteobacteria	22%			Chromatiales	21%			Chromatiaceae	17%	Halochromatium	10%
1	A51.3.1	Bacteria	100%	Firmicutes	23%	Clostridia	23%			Halanaerobiales	14%			Halanaerobiaceae	11%	Halothermothrix	9%
1	A91.3.1	Bacteria	100%	Firmicutes	30%	Clostridia	28%			Halanaerobiales	12%			Halanaerobiaceae	12%	Halothermothrix	11%
3	A11-3.2.5	Bacteria	100%	Proteobacteria	53%	Deltaproteobacteria	44%			Desulfurellales	14%			Desulfurellaceae	14%	Hippea	14%
3	A13.2.8	Bacteria	100%	Proteobacteria	56%	Deltaproteobacteria	47%			Desulfurellales	23%			Desulfurellaceae	23%	Hippea	23%
3	A43.2.2.2	Bacteria	100%	Proteobacteria	65%	Deltaproteobacteria	44%			Desulfurellales	17%			Desulfurellaceae	17%	Hippea	17%
1	A61.2.1	Bacteria	100%	Proteobacteria	54%	Deltaproteobacteria	30%			Desulfurellales	22%			Desulfurellaceae	22%	Hippea	22%
1	A10-1.3.5	Bacteria	100%	Acidobacteria	30%	Acidobacteria	30%			Acidobacteriales	30%			Acidobacteriaceae	30%	Holophaga	16%
2	A12.1.5	Bacteria	100%	Acidobacteria	57%	Acidobacteria	57%			Acidobacteriales	57%			Acidobacteriaceae	57%	Holophaga	50%
1	A91.2.4	Bacteria	100%	Acidobacteria	34%	Acidobacteria	34%			Acidobacteriales	34%			Acidobacteriaceae	34%	Holophaga	20%
2	A92.3.5	Bacteria	100%	Acidobacteria	63%	Acidobacteria	63%			Acidobacteriales	63%			Acidobacteriaceae	63%	Holophaga	41%
1	A61.2.2	Bacteria	99%	Proteobacteria	39%	Gammaproteobacteria	14%			Xanthomonadales	8%			Xanthomonadaceae	8%	Hydrocarboniphaga	8%
3	A93.1.1	Bacteria	100%	Proteobacteria	31%	Gammaproteobacteria	22%			Xanthomonadales	19%			Xanthomonadaceae	19%	Hydrocarboniphaga	12%
1	A91.3.2	Bacteria	86%	Aquificae	26%	Aquificae	26%			Aquificales	26%			Aquificaceae	24%	Hydrogenivirga	21%
N/A	SI5.2.1	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	100%			Burkholderiales	100%			Comamonadaceae	100%	Hydrogenophaga	98%
2	A12.2.5	Bacteria	96%	Proteobacteria	88%	Betaproteobacteria	72%			Burkholderiales	62%			Comamonadaceae	34%	Hylemonella	22%
3	A10-3.1.1	Bacteria	100%	Proteobacteria	100%	Alphaproteobacteria	100%			Rhizobiales	100%			Hyphomicrobiaceae	100%	Hyphomicrobium	91%
2	A42.1.6	Bacteria	100%	Proteobacteria	100%	Alphaproteobacteria	100%			Rhizobiales	100%			Hyphomicrobiaceae	100%	Hyphomicrobium	57%
1	A11-1.1.4	Bacteria	100%	Planctomycetes	34%	Planctomycetacia	34%			Planctomycetales	34%			Planctomycetaceae	34%	Isosphaera	34%
2	A62.1.4	Bacteria	100%	Planctomycetes	35%	Planctomycetacia	35%			Planctomycetales	35%			Planctomycetaceae	35%	Isosphaera	35%
2	A12.2.1	Bacteria	100%	Proteobacteria	86%	Deltaproteobacteria	72%			Desulfovibrionales	28%			Desulfovibrionaceae	26%	Lawsonia	26%
1	A31.2.2.5	Bacteria	98%	Lentisphaerae	30%	Lentisphaerae	30%			Lentisphaerales	26%			Lentisphaeraceae	26%	Lentisphaera	26%
2	A52.1.5	Bacteria	99%	Lentisphaerae	33%	Lentisphaerae	33%			Lentisphaerales	20%			Lentisphaeraceae	20%	Lentisphaera	20%
3	A53.3.1	Bacteria	100%	Spirochaetes	41%	Spirochaetes	41%			Spirochaetales	41%			Leptospiraceae	34%	Leptonema	34%

Mesocosm Depth	Sample	Domain		Phylum		Class		Subclass		Order		Suborder		Family		Genus	
3	A10-3.3.7	Bacteria	100%	Actinobacteria	100%	Actinobacteria	100%	Actinobacteridae	100%	Actinomycetales	100%	Micromonosporineae	49%	Micromonosporaceae	49%	Longispora	45%
2	A11-2.2.1	Bacteria	100%	Proteobacteria	100%	Gammaproteobacteria	100%			Xanthomonadales	100%			Xanthomonadaceae	100%	Lysobacter	60%
3	A12-3.3.5	Bacteria	100%	Proteobacteria	100%	Gammaproteobacteria	96%			Xanthomonadales	93%			Xanthomonadaceae	93%	Lysobacter	49%
3	A11-3.3.2	Bacteria	97%	Firmicutes	28%	Clostridia	25%			Thermoanaerobacteriales	13%			Thermoanaerobacteriacea	9%	Mahella	7%
2	A10-2.2.1	Bacteria	99%	Proteobacteria	46%	Deltaproteobacteria	38%			Desulfuromonales	18%			Desulfuromonaceae	15%	Malonomonas	15%
2	A10-2.2.5	Bacteria	100%	Proteobacteria	54%	Deltaproteobacteria	45%			Desulfuromonales	24%			Desulfuromonaceae	18%	Malonomonas	18%
2	A22.2.3.3	Bacteria	100%	Proteobacteria	60%	Deltaproteobacteria	29%			Desulfuromonales	16%			Desulfuromonaceae	15%	Malonomonas	15%
1	A31.2.3.2	Bacteria	100%	Proteobacteria	86%	Deltaproteobacteria	84%			Desulfuromonales	35%			Desulfuromonaceae	33%	Malonomonas	20%
2	A62.3.2	Bacteria	82%	Proteobacteria	39%	Deltaproteobacteria	14%			Desulfuromonales	6%			Desulfuromonaceae	6%	Malonomonas	6%
1	A31.2.3.3	Bacteria	100%	Deinococcus-Thermus	34%	Deinococci	34%			Thermales	34%			Thermaceae	34%	Marinithermus	26%
2	A52.1.3	Bacteria	99%	Deinococcus-Thermus	23%	Deinococci	23%			Thermales	23%			Thermaceae	23%	Marinithermus	16%
2	A32.2.1.1	Bacteria	100%	Proteobacteria	100%	Alphaproteobacteria	100%			Rhizobiales	100%			Phyllobacteriaceae	96%	Mesorhizobium	71%
1	A91.1.2	Bacteria	100%	Proteobacteria	100%	Gammaproteobacteria	74%			Methylococcales	33%			Methylococcaceae	33%	Methylococcus	32%
1	A51.1.4	Bacteria	100%	Actinobacteria	98%	Actinobacteria	98%	Actinobacteridae	98%	Actinomycetales	97%	Propionibacterineae	88%	Propionibacteriaceae	72%	Microlunatus	60%
N/A	SI6.2.5	Bacteria	100%	Actinobacteria	100%	Actinobacteria	100%	Actinobacteridae	100%	Actinomycetales	100%	Propionibacterineae	100%	Propionibacteriaceae	86%	Microlunatus	86%
1	A21.2.1.1	Bacteria	100%	Proteobacteria	100%	Alphaproteobacteria	100%			Rhizobiales	93%			Methylobacteriaceae	48%	Microvirga	48%
3	A53.1.3	Bacteria	100%	Proteobacteria	100%	Alphaproteobacteria	100%			Rhizobiales	87%			Methylobacteriaceae	62%	Microvirga	62%
1	A31.2.2.3	Bacteria	100%	Actinobacteria	100%	Actinobacteria	100%	Actinobacteridae	100%	Actinomycetales	100%	Corynebacterineae	98%	Mycobacteriaceae	94%	Mycobacterium	94%
N/A	SI6.3.4	Bacteria	100%	Proteobacteria	100%	Gammaproteobacteria	56%			Oceanospirillales	26%			Oceanospirillaceae	12%	Neptunomonas	6%
2	A12.1.4	Bacteria	99%	Proteobacteria	62%	Gammaproteobacteria	30%			Chromatiales	17%			Ectothiorhodospiraceae	15%	Nitrococcus	6%
2	A12.3.4	Bacteria	100%	Proteobacteria	99%	Gammaproteobacteria	65%			Chromatiales	24%			Ectothiorhodospiraceae	13%	Nitrococcus	5%
1	A51.2.8	Bacteria	99%	Proteobacteria	87%	Gammaproteobacteria	77%			Chromatiales	43%			Chromatiaceae	38%	Nitrosococcus	15%
3	A63.3.5	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	100%			Nitrosomonadales	100%			Nitrosomonadaceae	100%	Nitrosospira	100%
2	A10-2.1.2	Bacteria	100%	Nitrospira	100%	Nitrospira	100%			Nitrospirales	100%			Nitrospiraceae	100%	Nitrospira	100%
1	A11-1.2.3	Bacteria	100%	Nitrospira	99%	Nitrospira	99%			Nitrospirales	99%			Nitrospiraceae	99%	Nitrospira	99%
3	A11-3.3.3	Bacteria	100%	Nitrospira	100%	Nitrospira	100%			Nitrospirales	100%			Nitrospiraceae	100%	Nitrospira	100%
1	A12-1.1.5	Bacteria	100%	Nitrospira	28%	Nitrospira	28%			Nitrospirales	28%			Nitrospiraceae	28%	Nitrospira	28%
2	A22.2.2.4	Bacteria	100%	Nitrospira	100%	Nitrospira	100%			Nitrospirales	100%			Nitrospiraceae	100%	Nitrospira	100%
3	A53.1.2	Bacteria	100%	Nitrospira	100%	Nitrospira	100%			Nitrospirales	100%			Nitrospiraceae	100%	Nitrospira	100%
1	A91.3.3	Bacteria	100%	Nitrospira	100%	Nitrospira	100%			Nitrospirales	100%			Nitrospiraceae	100%	Nitrospira	100%
2	A12-2.1.3	Bacteria	100%	Actinobacteria	100%	Actinobacteria	100%	Actinobacteridae	100%	Actinomycetales	100%	Propionibacterineae	99%	Nocardioidaceae	99%	Nocardioides	98%
2	A12-2.1.4	Bacteria	100%	Proteobacteria	36%	Alphaproteobacteria	21%			Rickettsiales	14%			Incertae sedis 4	14%	Odyssella	14%
3	A12-3.3.1	Bacteria	100%	Proteobacteria	100%	Alphaproteobacteria	98%			Rickettsiales	63%			Incertae sedis 4	63%	Odyssella	62%
2	A42.1.5	Bacteria	100%	Proteobacteria	100%	Alphaproteobacteria	99%			Rickettsiales	63%			Incertae sedis 4	63%	Odyssella	61%
N/A	SI6.1.1	Bacteria	100%	Proteobacteria	93%	Alphaproteobacteria	75%			Rickettsiales	36%			Incertae sedis 4	26%	Odyssella	26%
2	A32.2.2.5	Bacteria	100%	Genera_incertae_sedis_OP10	99%											OP10	99%
3	A53.2.3	Bacteria	100%	Genera_incertae_sedis_OP10	79%											OP10	79%
2	A11-2.1.3	Bacteria	99%	Genera_incertae_sedis_OP11	95%											OP11	95%
2	A82.3.2	Bacteria	100%	Proteobacteria	96%	Betaproteobacteria	66%			Burkholderiales	56%			Comamonadaceae	24%	Ottowia	8%
1	A11-1.2.4	Bacteria	100%	Proteobacteria	56%	Alphaproteobacteria	30%			Rhodobacterales	12%			Rhodobacteraceae	12%	Pannonibacter	5%
1	A21.2.1.3	Bacteria	100%	Firmicutes	40%	Clostridia	30%			Clostridiales	14%			Acidaminococcaceae	5%	Papillibacter	5%
2	A72.1.5	Bacteria	98%	Proteobacteria	52%	Betaproteobacteria	30%			Burkholderiales	21%			Burkholderiaceae	12%	Paucimonas	6%
1	A71.3.5	Bacteria	100%	Proteobacteria	91%	Alphaproteobacteria	89%			Rhizobiales	86%			Hyphomicrobiaceae	69%	Pedomicrobium	23%
1	A51.2.7	Bacteria	100%	Proteobacteria	100%	Deltaproteobacteria	100%			Desulfuromonales	100%			Desulfuromonaceae	89%	Pelobacter	85%
3	A63.2.3	Bacteria	100%	Proteobacteria	100%	Deltaproteobacteria	100%			Desulfuromonales	97%			Desulfuromonaceae	97%	Pelobacter	58%
1	A11.2.2.3	Bacteria	100%	Firmicutes	87%	Clostridia	83%			Clostridiales	54%			Peptococcaceae	26%	Pelotomaculum	26%
3	A43.2.1.5	Bacteria	100%	Proteobacteria	44%	Deltaproteobacteria	26%			Bdellovibrionales	16%			Bacteriovoracaceae	15%	Peredibacter	15%
2	A72.1.2	Bacteria	100%	Proteobacteria	53%	Deltaproteobacteria	50%			Bdellovibrionales	20%			Bacteriovoracaceae	20%	Peredibacter	20%
2	A72.3.1	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	100%			Burkholderiales	77%			Alcaligenaceae	25%	Pigmentiphaga	24%
2	A12-2.3.2	Bacteria	100%	Planctomycetes	100%	Planctomycetacia	100%			Planctomycetales	100%			Planctomycetaceae	100%	Planctomyces	99%
1	A11.2.2.5	Bacteria	100%	Proteobacteria	82%	Deltaproteobacteria	78%			Myxococcales	70%	Nannocystineae	66%	Nannocystaceae	40%	Plesiocystis	33%
1	A71.2.1	Bacteria	100%	Proteobacteria	100%	Alphaproteobacteria	100%			Rhizobiales	100%			Phyllobacteriaceae	98%	Pseudaminobacter	62%

Mesocosm Depth	Sample	Domain		Phylum		Class		Subclass		Order		Suborder		Family		Genus	
2	A22.2.2.1	Bacteria	100%	Proteobacteria	100%	Gammaproteobacteria	97%			Xanthomonadales	93%			Xanthomonadaceae	93%	Pseudoxanthomonas	43%
2	A82.2.5	Bacteria	100%	Proteobacteria	48%	Gammaproteobacteria	25%			Chromatiales	22%			Chromatiaceae	20%	Rhabdochromatium	7%
N/A	SI5.1.2	Bacteria	99%	Proteobacteria	99%	Alphaproteobacteria	97%			Rhodospirillales	65%			Rhodospirillaceae	65%	Rhodocista	48%
3	A93.3.2	Bacteria	100%	Proteobacteria	100%	Alphaproteobacteria	100%			Rhizobiales	100%			Hyphomicrobiaceae	64%	Rhodoplanes	64%
2	A11-2.3.4	Bacteria	97%	Proteobacteria	31%	Alphaproteobacteria	12%			Rhodospirillales	6%			Acetobacteraceae	5%	Rhodovarius	4%
1	A10-1.2.2	Bacteria	100%	Bacteroidetes	97%	Flavobacteria	35%			Flavobacteriales	35%			Flavobacteriaceae	25%	Robiginitalea	9%
3	A12-3.1.2	Bacteria	100%	Bacteroidetes	98%	Flavobacteria	41%			Flavobacteriales	41%			Flavobacteriaceae	37%	Robiginitalea	14%
1	A11-1.1.3	Bacteria	100%	Chloroflexi	38%	Chloroflexi	30%			Chloroflexales	30%			Chloroflexaceae	30%	Roseiflexus	30%
1	A61.1.2	Bacteria	94%	Chloroflexi	23%	Chloroflexi	23%			Chloroflexales	23%			Chloroflexaceae	23%	Roseiflexus	23%
N/A	SI5.3.2	Bacteria	99%	Chloroflexi	41%	Chloroflexi	41%			Chloroflexales	41%			Chloroflexaceae	41%	Roseiflexus	41%
2	A12.1.3	Bacteria	100%	Proteobacteria	56%	Alphaproteobacteria	55%			Rhodobacterales	55%			Rhodobacteraceae	55%	Roseivirga	55%
2	A12.2.3	Bacteria	100%	Proteobacteria	53%	Alphaproteobacteria	53%			Rhodobacterales	53%			Rhodobacteraceae	53%	Roseivirga	53%
1	A12-1.1.4	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	100%			Burkholderiales	100%			Incertae sedis 5	82%	Rubrivivax	41%
2	A32.2.3.3	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	100%			Burkholderiales	99%			Incertae sedis 5	72%	Rubrivivax	41%
N/A	SI6.1.2	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	100%			Burkholderiales	99%			Incertae sedis 5	76%	Rubrivivax	32%
3	A11-3.2.4	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	99%			Burkholderiales	88%			Incertae sedis 5	76%	Schlegelella	43%
3	A13.1.8	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	96%			Burkholderiales	77%			Incertae sedis 5	34%	Schlegelella	29%
2	A32.2.3.2	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	99%			Burkholderiales	96%			Incertae sedis 5	67%	Schlegelella	23%
1	A51.1.5	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	97%			Burkholderiales	77%			Incertae sedis 5	50%	Schlegelella	40%
1	A51.2.6	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	100%			Burkholderiales	98%			Incertae sedis 5	58%	Schlegelella	29%
2	A52.1.2	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	100%			Burkholderiales	100%			Incertae sedis 5	46%	Schlegelella	26%
1	A71.1.3	Bacteria	100%	Proteobacteria	95%	Betaproteobacteria	91%			Burkholderiales	73%			Incertae sedis 5	45%	Schlegelella	40%
3	A73.2.2	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	100%			Burkholderiales	97%			Incertae sedis 5	56%	Schlegelella	41%
2	A82.3.4	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	97%			Burkholderiales	84%			Incertae sedis 5	44%	Schlegelella	35%
2	A92.2.2	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	92%			Burkholderiales	74%			Incertae sedis 5	53%	Schlegelella	41%
2	A92.2.3	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	91%			Burkholderiales	77%			Incertae sedis 5	53%	Schlegelella	37%
3	A93.1.5	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	98%			Burkholderiales	88%			Incertae sedis 5	57%	Schlegelella	43%
3	A93.2.3	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	100%			Burkholderiales	99%			Incertae sedis 5	52%	Schlegelella	28%
3	A63.1.4	Bacteria	100%	Actinobacteria	100%	Actinobacteria	100%	Actinobacteridae	100%	Actinomycetales	99%	Corynebacterineae	82%	Gordoniaceae	55%	Skermania	55%
1	A11.2.3.1	Bacteria	100%	Proteobacteria	99%	Deltaproteobacteria	98%			Syntrophobacterales	60%			Syntrophaceae	36%	Smithella	34%
1	A11.2.3.4	Bacteria	100%	Proteobacteria	85%	Deltaproteobacteria	77%			Syntrophobacterales	40%			Syntrophaceae	30%	Smithella	20%
1	A21.2.3.2	Bacteria	100%	Proteobacteria	99%	Deltaproteobacteria	99%			Syntrophobacterales	52%			Syntrophaceae	38%	Smithella	31%
1	A21.2.3.3	Bacteria	100%	Proteobacteria	97%	Deltaproteobacteria	97%			Syntrophobacterales	45%			Syntrophaceae	32%	Smithella	21%
3	A33.1.3	Bacteria	100%	Proteobacteria	91%	Deltaproteobacteria	88%			Syntrophobacterales	39%			Syntrophaceae	27%	Smithella	21%
2	A42.3.8	Bacteria	100%	Proteobacteria	100%	Deltaproteobacteria	100%			Syntrophobacterales	53%			Syntrophaceae	38%	Smithella	35%
2	A82.1.2	Bacteria	100%	Proteobacteria	95%	Deltaproteobacteria	95%			Syntrophobacterales	38%			Syntrophaceae	28%	Smithella	25%
2	A10-2.3.3	Bacteria	100%	Actinobacteria	48%	Actinobacteria	48%	Rubrobacteridae	35%	Rubrobacterales	35%	Rubrobacterineae	35%	Rubrobacteraceae	35%	Solirubrobacter	21%
1	A11-1.3.4	Bacteria	100%	Actinobacteria	73%	Actinobacteria	73%	Rubrobacteridae	64%	Rubrobacterales	64%	Rubrobacterineae	64%	Rubrobacteraceae	64%	Solirubrobacter	37%
3	A13.3.8	Bacteria	100%	Actinobacteria	75%	Actinobacteria	75%	Rubrobacteridae	64%	Rubrobacterales	64%	Rubrobacterineae	64%	Rubrobacteraceae	64%	Solirubrobacter	49%
2	A42.3.6	Bacteria	100%	Actinobacteria	93%	Actinobacteria	93%	Rubrobacteridae	89%	Rubrobacterales	89%	Rubrobacterineae	89%	Rubrobacteraceae	89%	Solirubrobacter	80%
1	A51.2.3	Bacteria	100%	Actinobacteria	100%	Actinobacteria	100%	Rubrobacteridae	100%	Rubrobacterales	100%	Rubrobacterineae	100%	Rubrobacteraceae	100%	Solirubrobacter	50%
3	A63.2.5	Bacteria	99%	Actinobacteria	62%	Actinobacteria	62%	Rubrobacteridae	45%	Rubrobacterales	45%	Rubrobacterineae	45%	Rubrobacteraceae	45%	Solirubrobacter	26%
2	A92.3.3	Bacteria	100%	Actinobacteria	90%	Actinobacteria	90%	Rubrobacteridae	87%	Rubrobacterales	87%	Rubrobacterineae	87%	Rubrobacteraceae	87%	Solirubrobacter	76%
1	A71.3.3	Bacteria	100%	Proteobacteria	100%	Alphaproteobacteria	100%			Sphingomonadales	98%			Sphingomonadaceae	98%	Sphingopyxis	47%
3	A73.1.4	Bacteria	99%	Firmicutes	29%	Clostridia	25%			Clostridiales	15%			Clostridiaceae	8%	Sporobacter	7%
1	A11.2.1.2	Bacteria	100%	Proteobacteria	69%	Deltaproteobacteria	41%			Myxococcales	24%	Cystobacterineae	21%	Cystobacteraceae	20%	Stigmatella	13%
3	A11-3.3.4	Bacteria	100%	Proteobacteria	100%	Deltaproteobacteria	100%			Myxococcales	100%	Cystobacterineae	100%	Cystobacteraceae	100%	Stigmatella	31%
2	A12.2.2	Bacteria	100%	Proteobacteria	48%	Deltaproteobacteria	43%			Myxococcales	23%	Cystobacterineae	23%	Cystobacteraceae	16%	Stigmatella	7%
3	A43.2.3.3	Bacteria	100%	Proteobacteria	76%	Deltaproteobacteria	70%			Myxococcales	42%	Cystobacterineae	36%	Cystobacteraceae	29%	Stigmatella	18%
3	A63.3.4	Bacteria	100%	Proteobacteria	99%	Deltaproteobacteria	98%			Myxococcales	45%	Cystobacterineae	44%	Cystobacteraceae	44%	Stigmatella	24%
1	A71.2.4	Bacteria	100%	Proteobacteria	69%	Deltaproteobacteria	32%			Myxococcales	20%	Cystobacterineae	18%	Cystobacteraceae	10%	Stigmatella	9%
3	A23.2.5	Bacteria	93%	Proteobacteria	29%	Gammaproteobacteria	11%			Aeromonadales	3%			Succinivibrionaceae	2%	Succinivibrio	2%
1	A71.1.4	Bacteria	99%	Proteobacteria	38%	Epsilonproteobacteria	21%			Campylobacterales	16%			Helicobacteraceae	16%	Sulfurovum	16%

Mesocosm Depth	Sample	Domain		Phylum		Class		Subclass		Order		Suborder		Family		Genus	
2	A11-2.2.4	Bacteria	100%	Proteobacteria	33%	Betaproteobacteria	15%			Burkholderiales	11%			Alcaligenaceae	8%	Sutterella	8%
3	A83.1.1	Bacteria	95%	Proteobacteria	88%	Alphaproteobacteria	81%			Rhodospirillales	57%			Acetobacteraceae	49%	Swaminathania	21%
2	A32.2.1.3	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	100%			Burkholderiales	100%			Incertae sedis 5	54%	Tepidimonas	39%
3	A83.3.2	Bacteria	100%	Proteobacteria	99%	Betaproteobacteria	99%			Burkholderiales	98%			Incertae sedis 5	77%	Tepidimonas	70%
3	A11-3.1.4	Bacteria	100%	Firmicutes	58%	Clostridia	57%			Thermoanaerobacteriales	28%			Thermoanaerobacteriaceae	28%	Thermanaeromonas	24%
1	A71.2.5	Bacteria	98%	Firmicutes	34%	Clostridia	34%			Thermoanaerobacteriales	16%			Thermoanaerobacteriaceae	14%	Thermanaeromonas	10%
3	A53.3.5	Bacteria	100%	Firmicutes	39%	Clostridia	39%			Thermoanaerobacteriales	25%			Thermodesulfobiaceae	24%	Thermodesulfobium	24%
2	A92.3.4	Bacteria	98%	Firmicutes	47%	Clostridia	45%			Thermoanaerobacteriales	27%			Thermodesulfobiaceae	26%	Thermodesulfobium	26%
1	A81.3.2	Bacteria	97%	Proteobacteria	45%	Deltaproteobacteria	39%			Syntrophobacterales	36%			Syntrophobacteraceae	35%	Thermodesulforhabdus	15%
1	A91.1.3	Bacteria	100%	Firmicutes	51%	Clostridia	36%			Clostridiales	33%			Clostridiaceae	25%	Thermohalobacter	17%
3	A33.2.3.1	Bacteria	100%	Actinobacteria	59%	Actinobacteria	59%	Rubrobacteridae	53%	Rubrobacterales	53%	Rubrobacterineae	53%	Rubrobacteraceae	53%	Thermoleophilum	27%
1	A11.2.2.4	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	100%			Burkholderiales	60%			Incertae sedis 5	33%	Thiobacter	27%
3	A12-3.2.3	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	98%			Burkholderiales	84%			Incertae sedis 5	67%	Thiobacter	36%
3	A33.2.2.5	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	97%			Burkholderiales	85%			Incertae sedis 5	43%	Thiobacter	40%
2	A42.1.7	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	100%			Burkholderiales	74%			Incertae sedis 5	66%	Thiobacter	60%
2	A52.2.5	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	98%			Burkholderiales	49%			Incertae sedis 5	38%	Thiobacter	38%
3	A83.3.4	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	100%			Burkholderiales	87%			Incertae sedis 5	71%	Thiobacter	43%
2	A92.1.3	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	98%			Burkholderiales	89%			Incertae sedis 5	61%	Thiobacter	34%
3	A93.2.1	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	100%			Burkholderiales	97%			Incertae sedis 5	53%	Thiobacter	39%
N/A	SI6.3.5	Bacteria	100%	Proteobacteria	100%	Betaproteobacteria	100%			Burkholderiales	68%			Incertae sedis 5	54%	Thiobacter	47%
1	A61.1.5	Bacteria	99%	Proteobacteria	58%	Gammaproteobacteria	21%			Chromatiales	11%			Chromatiaceae	10%	Thiorhodococcus	4%
1	A11-1.1.6	Bacteria	100%	Proteobacteria	99%	Gammaproteobacteria	96%			Chromatiales	45%			Ectothiorhodospiraceae	38%	Thiorhodospira	36%
1	A21.2.2.2	Bacteria	100%	Proteobacteria	100%	Gammaproteobacteria	95%			Chromatiales	51%			Ectothiorhodospiraceae	30%	Thiorhodospira	27%
3	A43.2.3.1	Bacteria	100%	Proteobacteria	98%	Gammaproteobacteria	83%			Chromatiales	38%			Ectothiorhodospiraceae	20%	Thiorhodospira	17%
3	A63.1.3	Bacteria	100%	Proteobacteria	100%	Gammaproteobacteria	98%			Chromatiales	51%			Ectothiorhodospiraceae	33%	Thiorhodospira	22%
2	A82.3.1	Bacteria	100%	Proteobacteria	47%	Gammaproteobacteria	38%			Chromatiales	27%			Ectothiorhodospiraceae	22%	Thiorhodospira	12%
1	A91.1.1	Bacteria	100%	Proteobacteria	99%	Gammaproteobacteria	81%			Chromatiales	32%			Ectothiorhodospiraceae	23%	Thiorhodospira	22%
2	A10-2.3.1	Bacteria	100%	Proteobacteria	56%	Alphaproteobacteria	27%			Rhodospirillales	23%			Rhodospirillaceae	15%	Tistrella	15%
1	A31.2.1.4	Bacteria	100%	Proteobacteria	90%	Alphaproteobacteria	54%			Rhodospirillales	51%			Rhodospirillaceae	33%	Tistrella	31%
3	A63.1.5	Bacteria	94%	Proteobacteria	40%	Alphaproteobacteria	15%			Rhodospirillales	11%			Rhodospirillaceae	11%	Tistrella	11%
3	A83.1.4	Bacteria	100%	Proteobacteria	100%	Alphaproteobacteria	100%			Rhodospirillales	86%			Rhodospirillaceae	77%	Tistrella	66%
3	A13.3.7	Bacteria	100%	Proteobacteria	96%	Deltaproteobacteria	96%			Desulfuromonales	71%			Geobacteraceae	64%	Trichlorobacter	64%
1	A21.2.1.2	Bacteria	99%	Proteobacteria	85%	Deltaproteobacteria	78%			Desulfuromonales	30%			Geobacteraceae	26%	Trichlorobacter	20%
N/A	SI6.1.3	Bacteria	100%	Proteobacteria	46%	Deltaproteobacteria	17%			Desulfuromonales	7%			Geobacteraceae	7%	Trichlorobacter	7%
2	A10-2.1.3	Bacteria	88%	Proteobacteria	38%	Betaproteobacteria	16%			Burkholderiales	7%			Comamonadaceae	6%	Variovorax	2%
1	A10-1.3.3	Bacteria	100%	Verrucomicrobia	62%	Verrucomicrobiae	62%			Verrucomicrobiales	62%			Verrucomicrobiaceae	40%	Verrucomicrobium	40%
3	A12-3.2.2	Bacteria	100%	Verrucomicrobia	57%	Verrucomicrobiae	57%			Verrucomicrobiales	57%			Verrucomicrobiaceae	39%	Verrucomicrobium	39%
3	A12-3.2.5	Bacteria	100%	Verrucomicrobia	27%	Verrucomicrobiae	27%			Verrucomicrobiales	27%			Verrucomicrobiaceae	27%	Verrucomicrobium	27%
1	A31.2.2.2	Bacteria	100%	Verrucomicrobia	90%	Verrucomicrobiae	90%			Verrucomicrobiales	90%			Verrucomicrobiaceae	88%	Verrucomicrobium	88%
1	A41.2.6	Bacteria	100%	Verrucomicrobia	68%	Verrucomicrobiae	68%			Verrucomicrobiales	68%			Verrucomicrobiaceae	45%	Verrucomicrobium	45%
2	A42.2.8	Bacteria	100%	Verrucomicrobia	68%	Verrucomicrobiae	68%			Verrucomicrobiales	68%			Verrucomicrobiaceae	28%	Verrucomicrobium	28%
2	A52.3.4	Bacteria	100%	Verrucomicrobia	52%	Verrucomicrobiae	52%			Verrucomicrobiales	52%			Verrucomicrobiaceae	47%	Verrucomicrobium	47%
2	A72.2.3	Bacteria	100%	Verrucomicrobia	78%	Verrucomicrobiae	78%			Verrucomicrobiales	78%			Verrucomicrobiaceae	50%	Verrucomicrobium	50%
2	A72.3.4	Bacteria	100%	Verrucomicrobia	82%	Verrucomicrobiae	82%			Verrucomicrobiales	82%			Verrucomicrobiaceae	74%	Verrucomicrobium	72%
2	A92.3.2	Bacteria	100%	Verrucomicrobia	82%	Verrucomicrobiae	82%			Verrucomicrobiales	82%			Verrucomicrobiaceae	71%	Verrucomicrobium	71%
2	A10-2.1.1	Bacteria	100%	Lentisphaerae	28%	Lentisphaerae	28%			Victivallales	27%			Victivallaceae	27%	Victivallis	27%
3	A73.3.2	Bacteria	99%	Chlamydiae	18%	Chlamydiae	18%			Chlamydiales	18%			Waddliaceae	10%	Waddlia	10%
2	A11-2.1.4	Bacteria	84%	Proteobacteria	34%	Alphaproteobacteria	14%			Rickettsiales	10%			Anaplasmataceae	10%	Xenohaliotis	9%
2	A42.2.7	Bacteria	100%	Verrucomicrobia	98%	Verrucomicrobiae	98%			Verrucomicrobiales	98%			Xiphinematobacteriaceae	77%	Xiphinematobacter	77%
N/A	SI6.3.3	Bacteria	100%	Verrucomicrobia	75%	Verrucomicrobiae	75%			Verrucomicrobiales	75%			Xiphinematobacteriaceae	64%	Xiphinematobacter	64%
3	A23.2.3	Bacteria	100%	Actinobacteria	66%	Actinobacteria	66%	Actinobacteridae	45%	Actinomycetales	44%	Micrococcineae	35%	Promicromonosporaceae	14%	Xylanibacterium	13%
N/A	SI5.3.3	Bacteria	93%	Actinobacteria	21%	Actinobacteria	21%	Actinobacteridae	16%	Actinomycetales	13%	Micrococcineae	4%	Micrococcaceae	2%	Yania	2%
1	A91.1.4	Bacteria	100%	Proteobacteria	36%	Gammaproteobacteria	16%			Oceanospirillales	4%			Hahellaceae	3%	Zooshikella	3%

Appendix P. Primer Design, Electrophoresis, Quantifying DNA with Spectrophotometer, Cloning, Sequencing, and Sequence Analysis

Primer Design

The 16S rRNA gene sequence is about 1,550 bp long and is composed of both variable and conserved regions (Baker, Smith, & Cowan, 2003; Clarridge, 2004). The gene is large enough to provide distinguishing and statistically valid measurements. Choosing the correct primer is dependent on the research criteria. For example, if a specific genus of bacteria is being sought then only sequences unique to that bacteria are needed. However, for this study conserved sequences are required. Conserved sequences are sequences found in many bacterial species. Some conserved sequences are considered "universal" and can be found across specific sequences of 16S rDNA amongst all species of bacteria. Universal primers are usually chosen as complementary to the conserved regions at the beginning of the gene and at either the 540-bp region or at the end of the 16S sequence; the sequence of the variable region in between is used for the comparative taxonomy (Clarridge, 2004). However, research has shown that primers designed to be complementary to conserved regions of the groups present in the phylogenetic tree are not necessarily complementary to all those that exist in the database today (Baker, Smith, & Cowan, 2003). Most primers are 17-30 bp in length and separated by amplified region dictated by primer selection (Maier, Pepper, & Gerba, 2000).

As a rule of thumb, primers should generally be 16-24 nucleotides long with closely matched melting temperatures (less than 5 °C difference), avoid runs of identical nucleotides, avoid a 3'-end T, and have at least a 5 base match at the 3' end. The melting point of an oligonucleotide is the temperature at which it dissociates from a complementary sequence and is closely tied to the estimated annealing temperature. The annealing temperature dictates how efficiently the primer binds to the complementary target region. If it is too high the primer will

not anneal, too low and they will anneal to non-target regions. Both primers used should have similar melting temperatures (T_m). An estimate of melting temperature can be attained using the below equation (Marchesi, 2001):

$T_m = 4(G+C) + 2(A+T)$

The G+C content has a greater effect on melting temperature due to the three hydrogen bonds between these bases vice two between A+T (Pepper & Dowd, 2002), thus DNA having higher G+C content requires more energy (higher temperature) to denature.

Avoiding runs of identical nucleotides is necessary in order to ensure that the primers anneal at different sites on the DNA. If complementary bases are present in the primers then the researcher runs the risk of producing a "primer dimer" (S. A. Smith, 2005).

The use of degenerate primers may also improve amplifying the template DNA when the exact nucleotide sequence is unknown or contains mismatches to possible primers.

Because the position of the primers within a genome defines the size of the amplification product, this size can be compared to DNA standards using gel electrophoresis.

Electrophoresis

Gel electrophoresis is a technique used for viewing, sizing, and even quantifying DNA molecules. An agarose gel is made as outlined in Appendix E. DNA samples are loaded into prepared wells, along with a known standard (ladder) and negative control. Voltage is applied to the gel, which causes the DNA to migrate toward the positively charged anode due to the DNA's negatively charged phosphates. Larger DNA fragments of higher molecular weight in base pairs (bp) migrate slower than smaller ones, thus enabling separation of DNA fragments by size.

Ethidium bromide (EtBr) is a DNA intercolating agent, which binds to DNA and fluoresces under UV light; thus, allowing visualization of the DNA under ultraviolet (UV) light and comparison to ladders (standards of known size) that run parallel on the prepared gel (Maier,

Pepper, & Gerba, 2000). If bands are present in the correct location, this confirms that the specified organism or functional gene is present in the PCR product. The brightness of the band of the extracted DNA on the agarose gel also serves as an indication DNA concentration.

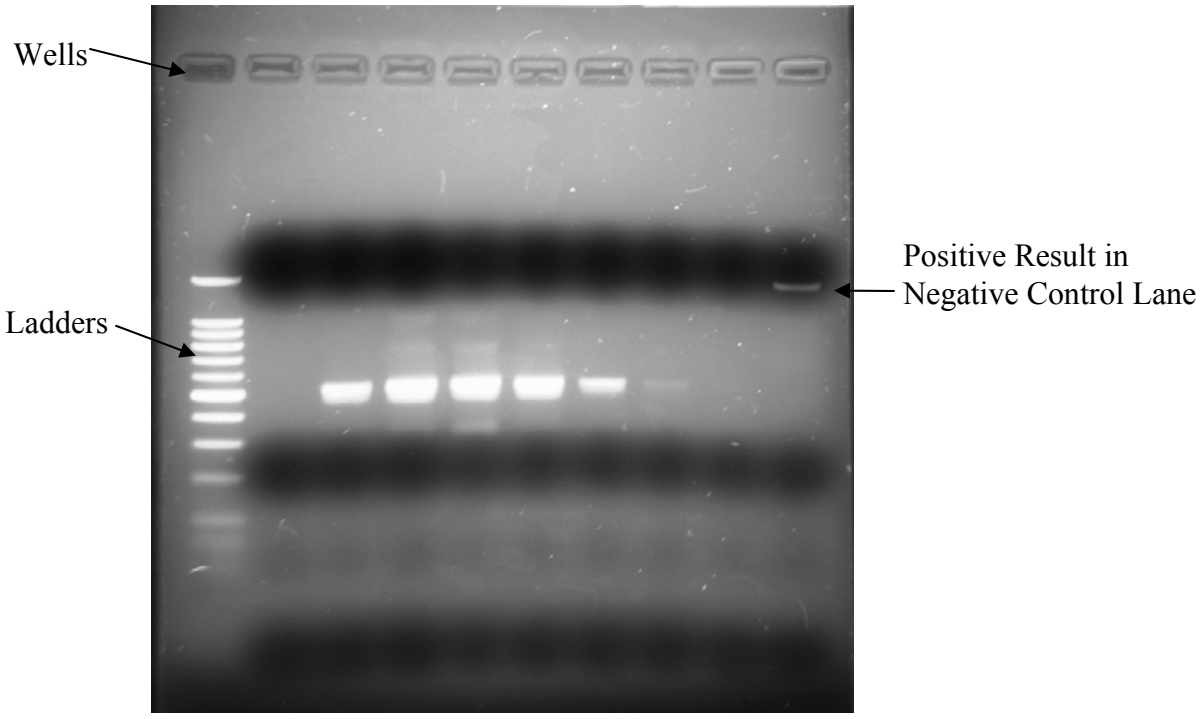

Figure 20. Agarose Gel Image of DNA. Left-most lane contains DNA ladder; right-most contains negative control showing positive results.

Quantifying DNA with Spectrophotometer

DNA purity and concentrations in the template DNA and amplified PCR product can be estimated using a spectrophotometer; this is a useful tool in ascertaining problems with PCR amplification and ensuring proper DNA concentration when loading samples or preparing reactions. UV wavelength absorbance ratios are used to determine ultimate DNA purity and concentrations. When DNA is extracted from samples, some protein typically remains in the DNA solution; protein is tightly bound to DNA and complete removal of protein is not always possible (S. A. Smith, 2005). Both protein and DNA absorb UV light, but they have different absorbance curves. DNA's peak absorbance is at 260 nm and protein's is at 280 nm. One can

calculate the purity of the DNA solution by determining the ratio of the absorbance; an A_{260}/A_{280} ratio of 1.7-2.0 and a A_{260}/A_{230} ratio of greater than 1.9 indicates a relatively pure DNA concentration (Manchester, 1995; S. A. Smith, 2005; Tsai & Rochelle, 2001).

If the solution is relatively free of protein, then one can take the absorbance at 260 nm as a measure for concentration of DNA by the formula:

1 A_{260} O.D. (optical density) Unit for dsDNA = 50 ng/µl

For example,

If a 1:100 dilution of dsDNA (5 µl DNA extract + 495 µl pure water) gives an A_{260} = 1.75;

[DNA] = 1.75x50= 87.5 ng/µl

Total mass of DNA = 87.5 ng/µl * 5 µl = 437.5 ng

Cloning

The product of PCR is a heterogeneous mixture of amplified 16S rDNA which is isolated through cloning. Gene cloning is the process of incorporating a DNA sequence into a cloning vector (plasmid), which can replicate in another organism. The plasmid carries genes for antibiotic resistance, and a DNA strand, which contains the gene of interest. Both are cut with the same restriction enzyme. Restriction enzymes such as EcoR1 surround the DNA molecule at the point it seeks (sequence GAATTC). It cuts one strand of the DNA double helix at one point and the second strand at a different, complementary point (between the G and the A base). The separated pieces have single stranded "sticky-ends," which allow the complementary pieces to combine. The plasmid is opened up and the gene is freed from its parent DNA strand. The opened plasmid and the freed gene are mixed with DNA ligase, which reforms the two pieces as recombinant DNA. This recombinant DNA mix is allowed to transform in *E. coli*

(transformation). The bacterial culture is then plated on a growth media exposed to antibiotics. All the cells except those which have incorporated the plasmid DNA recombinant are killed, leaving a cell culture containing the desired recombinant DNA. (National Health Museum, 1999)

X-gal is a sugar which, when metabolized by beta-galactosidase produced by the LacZ gene in the plasmid vector, produces a blue product (Maier, Pepper, & Gerba, 2000). Thus X-gal can also be used to visualize colonies that got the plasmid but no insert (blue colonies) and colonies that received plasmid plus DNA insert (white colonies).

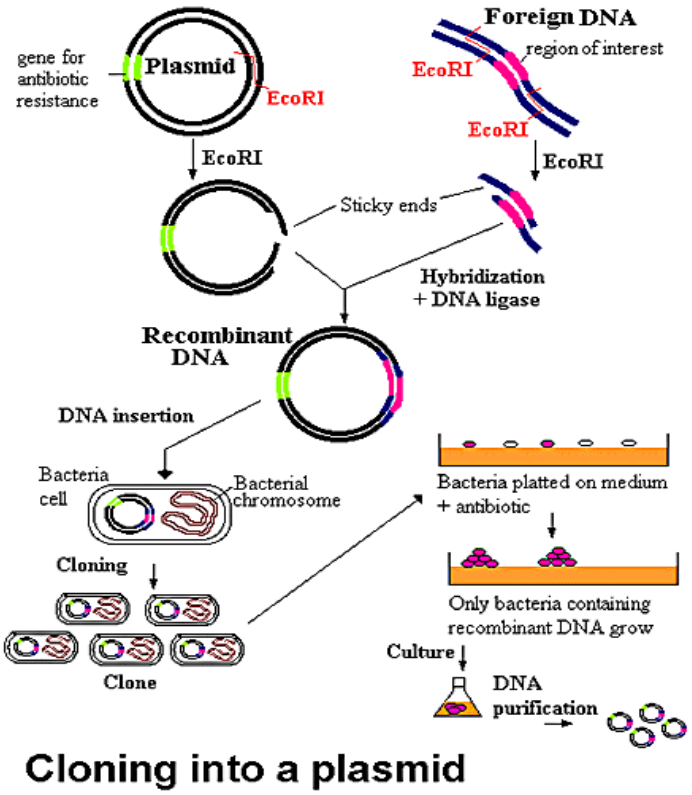

Figure 21. Cloning into a plasmid. (National Health Museum, 1999)

Purifying and Verifying DNA Insert

Following cloning, plasmids are purified and isolated using one of a variety of commercially available kits and restriction digestion conducted using a restriction enzyme such as EcoR1 in order to verify insertion of targeted DNA.

Sequencing

Following cloning, retrieval of the inserts of clones for DNA sequencing and identification using primers specific to the plasmid vector is done. Primers M13F or M13R are typically used, because these primers are specific to the cloned vector and are not universal primers. Thermocycling replicates the purified DNA isolate using a dNTP mix, dye terminators, and polymerase similar to PCR. The product is a mixture of fragments of varying lengths due to the addition of specially labeled bases called dye terminators, which randomly terminate the sequence. Each of the four added labeled terminator bases has different fluorescent dye, each of which absorbs at a different wavelength (Clarridge, 2004) allowing recognition during sequencing via capillary electrophoresis.

Sequence Analysis

Sequence analysis software judges the identity of the nucleotide at each position by comparing the relative heights of the peaks. If two peaks are overlapped, then the software is unable to judge what the nucleotide is, and an "N" (unknown) is shown in the position (see Fig.23). The following figures show examples of high and low quality sequence data. Other errors, which may need editing, include unseparated nucleotide spikes and long nucleotide runs.

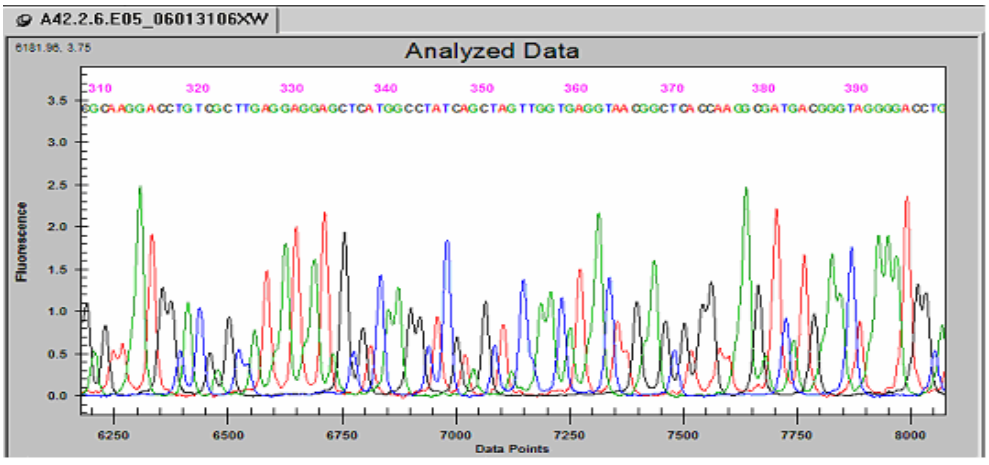

Figure 22. High quality chromatogram.

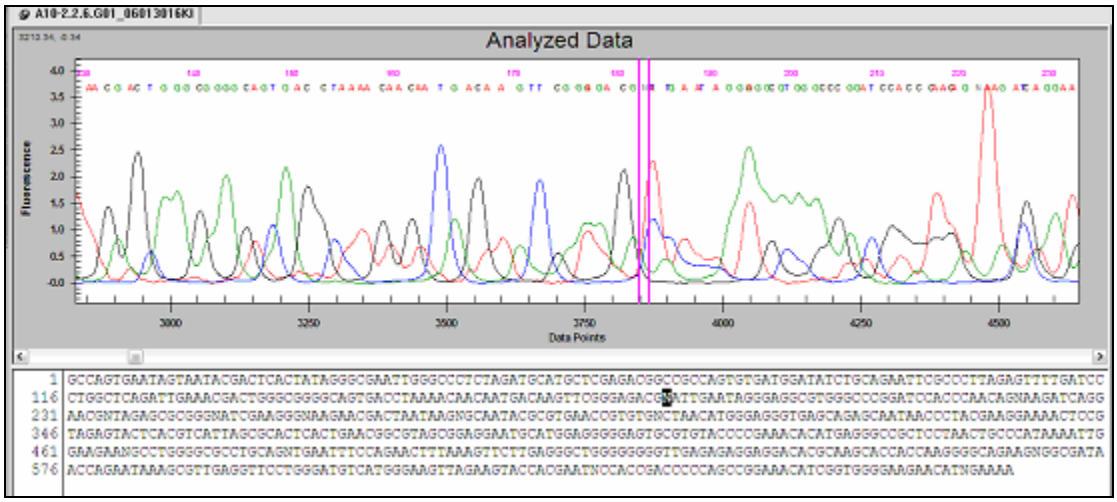

Figure 23. Low quality chromatogram showing unknown nucleotide "N".

Appendix Q. EstimateS Results

EstimateS (Version 7.5.0), Copyright R. K. Colwell: http://viceroy.eeb.uconn.edu/estimates
Diversity Output from Input File: LEVEL 1 (April 23, 2006)

Samples	Individuals (computed)	Sobs (Mao Tau)	Sobs 95% CI Lower Bound	Sobs 95% CI Upper Bound	Sobs SD (Mao Tau)	Singletons Mean	Doubletons Mean	Uniques Mean	Duplicates Mean	ACE Mean	Chao 1 Mean	Chao 1 95% CI Lower Bound	Chao 1 95% CI Upper Bound	Chao 1 SD (analytical)	Chao 2 Mean	Cole Rarefaction	Shannon Mean	Simpson Mean
1	10.17	9.92	8.14	11.69	0.9	9.82	0.26	10.08	0	58.14	53.23	23.89	147.28	27.71	54.14	10.11	2.28	I
2	20.33	19.77	16.25	23.3	1.8	20.28	0.42	20.68	0.04	227.49	187.21	92.68	407.46	74.9	207.64	20.12	3.02	I
3	30.5	29.57	24.31	34.82	2.68	29.44	0.76	30.1	0.14	441.97	351.84	190.03	679.19	118.82	415.08	30.02	3.39	I
4	40.67	39.3	32.33	46.27	3.56	38.48	1.2	39.48	0.26	698.53	478.38	275.12	858.47	143.14	668.65	39.81	3.67	I
5	50.83	48.98	40.31	57.65	4.42	47.48	1.64	48.68	0.56	906.02	581.09	350.58	988.99	157.66	886.75	49.52	3.88	I
6	61	58.59	48.24	68.94	5.28	57.08	1.96	58.42	0.82	1167.85	746.5	466.94	1218.45	186.53	1055.18	59.13	4.06	I
7	71.17	68.14	56.12	80.16	6.13	65.9	2.56	67.44	1.3	1247.74	851.29	549.23	1343.73	197.89	1143.77	68.64	4.21	972.57
8	81.33	77.64	63.96	91.31	6.98	74.92	3.06	76.58	1.76	1270.83	939.1	622	1441.43	204.65	1228.6	78.07	4.34	957.43
9	91.5	87.07	71.75	102.39	7.82	83.4	3.5	85.18	2.2	1387.75	1073.48	725.97	1610.38	221.37	1276.12	87.42	4.45	963.62
10	101.67	96.44	79.48	113.4	8.65	92.24	3.84	94.1	2.6	1372.99	1109.33	764.35	1632.66	217.68	1313.19	96.69	4.55	973.48
11	111.83	105.75	87.16	124.34	9.48	100.92	4.34	102.82	3.28	1312.91	1110.49	779.64	1603.9	206.92	1274.56	105.88	4.64	935.81
12	122	115	94.79	135.21	10.31	109	5	111	4	1252.86	1096	782.54	1556.64	194.55	1234.25		4.72	922.63

EstimateS (Version 7.5.0), Copyright R. K. Colwell: http://viceroy.eeb.uconn.edu/estimates
Diversity Output from Input File: Level 2 (April 23, 2006)

Samples	Individuals (computed)	Sobs (Mao Tau)	Sobs 95% CI Lower Bound	Sobs 95% CI Upper Bound	Sobs SD (Mao Tau)	Sobs Mean (runs)	Singletons Mean	Doubletons Mean	Uniques Mean	Duplicates Mean	ACE Mean	Chao 1 Mean	Chao 1 95% CI Lower Bound	Chao 1 95% CI Upper Bound	Chao 1 SD (analytical)	Chao 2 Mean	Cole Rarefaction	Shannon Mean	Simpson Mean
1	9.42	9.33	7.63	11.04	0.87	9.54	9.48	0.06	9.54	0	50.86	49.54	21.51	143.74	27.23	47.42	9.35	2.25	I
2	18.83	18.56	15.19	21.93	1.72	18.5	18.32	0.18	18.44	0.06	181.98	165.13	78.7	376.36	70.3	162.97	18.59	2.91	I
3	28.25	27.68	22.67	32.69	2.55	27.46	27.06	0.36	27.22	0.24	387.69	330.19	171.9	662.6	118.6	320.64	27.7	3.3	I
4	37.67	36.7	30.09	43.31	3.37	36.5	35.76	0.68	35.98	0.52	647.25	493.35	275.05	912.01	155.82	485.07	36.7	3.59	I
5	47.08	45.61	37.42	53.79	4.18	45.42	44.26	1.02	44.44	0.98	891.13	649.42	381.03	1133.07	185.35	617.88	45.59	3.8	I
6	56.5	54.41	44.68	64.14	4.96	54.26	52.54	1.56	52.82	1.44	1077.01	754.29	461.34	1258.58	197.48	721.54	54.38	3.98	I
7	65.92	63.11	51.85	74.36	5.74	62.94	60.6	2.18	60.88	2.06	1141.69	776.72	491.84	1251.15	188.73	732.79	63.05	4.13	I
8	75.33	71.7	58.94	84.45	6.51	71.46	68.32	2.9	68.58	2.88	1041.94	755.45	493.29	1180.73	171.31	692.76	71.63	4.25	I
9	84.75	80.18	65.95	94.42	7.26	79.98	76.04	3.44	76.22	3.76	1088.59	818.7	546.65	1249.46	175.53	689.78	80.11	4.36	877.23
10	94.17	88.56	72.86	104.26	8.01	88.56	83.58	4.36	83.74	4.82	937.1	771.72	528.87	1148.57	155.06	656.74	88.5	4.46	766.08
11	103.58	96.83	79.68	113.98	8.75	96.9	91.08	5.02	91.14	5.76	926.84	794.72	554.8	1160.38	151.76	662.95	96.79	4.55	737.68
12	113	105	86.41	123.59	9.49	105	98	6	98	7	883.28	784	557.18	1124.59	142.36	649.61		4.62	703.11

EstimateS (Version 7.5.0), Copyright R. K. Colwell: http://viceroy.eeb.uconn.edu/estimates
Diversity Output from Input File: Level 3 (April 23, 2006)

Samples	Individuals (computed)	Sobs (Mao Tau)	Sobs 95% CI Lower Bound	Sobs 95% CI Upper Bound	Sobs SD (Mao Tau)	Sobs Mean (runs)	Singletons Mean	Doubletons Mean	Uniques Mean	Duplicates Mean	ACE Mean	Chao 1 Mean	Chao 1 95% CI Lower Bound	Chao 1 95% CI Upper Bound	Chao 1 SD (analytical)	Chao 2 Mean	Cole Rarefaction	Shannon Mean	Simpson Mean
1	8.92	8.83	7.16	10.5	0.85	9.2	9.16	0.04	9.2	0	47.9	47.36	20.51	138.68	26.25	44.68	8.88	2.2	I
2	17.83	17.61	14.29	20.93	1.69	17.82	17.58	0.24	17.72	0.1	170.28	154.35	73.58	353.42	65.99	151.53	17.69	2.87	I
3	26.75	26.32	21.37	31.27	2.52	26.26	25.76	0.5	26.02	0.24	352.22	292.83	152.41	590.86	105.8	303.81	26.44	3.25	I
4	35.67	34.97	28.41	41.53	3.35	35	34.3	0.7	34.64	0.36	602.45	469.65	260.65	873.33	149.74	505.34	35.11	3.54	I
5	44.58	43.56	35.41	51.71	4.16	43.56	42.56	1	43.04	0.52	868.45	624.16	364.59	1094.72	179.81	720.04	43.72	3.76	I
6	53.5	52.09	42.36	61.82	4.96	52.04	50.66	1.38	51.28	0.76	1122.32	744.46	452.77	1249.56	197.24	944.82	52.25	3.94	I
7	62.42	60.56	49.27	71.85	5.76	60.56	58.76	1.8	59.44	1.12	1357.31	828.82	519.62	1346.84	205.48	1108.34	60.72	4.09	I
8	71.33	68.97	56.13	81.81	6.55	68.78	66.26	2.52	66.98	1.8	1359.51	855.95	553.11	1348.6	198.18	1095.66	69.11	4.22	I
9	80.25	77.32	62.94	91.69	7.33	76.8	73.72	3.08	74.5	2.3	1408.89	875.24	578.9	1346.7	191.68	1153.75	77.44	4.33	1430.86
10	89.17	85.61	69.7	101.51	8.11	84.92	81.14	3.78	82	2.92	1227.57	855.29	579.3	1285.5	176.63	1038.04	85.69	4.43	1257.67
11	98.08	93.83	76.41	111.26	8.89	93.48	89	4.48	89.94	3.54	1086.54	839.88	581.28	1235.64	163.92	960.17	93.88	4.52	1123.95
12	107	102	83.06	120.94	9.66	102	97	5	98	4	1091.4	878	617.78	1269.5	163.49	973.38		4.61	1134.2

EstimateS (Version 7.5.0), Copyright R. K. Colwell: http://viceroy.eeb.uconn.edu/estimates
Diversity Output from Input File: C. comosa (June 9, 2006)

Samples	Individuals (computed)	Sobs (Mao Tau)	Singletons Mean	Doubletons Mean	Uniques Mean	Duplicates Mean	ACE Mean	Chao 1 Mean	Chao 1 95% CI Lower Bound	Chao 1 95% CI Upper Bound	Cole Rarefaction	Shannon Mean	Simpson Mean
1	8.17	8	7.88	0.17	8.05	0	38.27	35.31	15.72	106.88	8.06	2.05	I
2	16.33	15.87	15.41	0.35	15.68	0.13	138.47	113.79	53.64	271.31	15.93	2.74	I
3	24.5	23.6	22.8	0.53	23.12	0.39	301.19	223.07	113.48	467.57	23.63	3.13	I
4	32.67	31.2	30.05	0.69	30.31	0.82	515.85	350.5	189.04	678.01	31.19	3.41	I
5	40.83	38.67	37.07	0.86	37.23	1.37	681.36	466.3	263.29	853.29	38.63	3.62	351.44
6	49	46	44	1	44	2	680.77	519	303.65	914.34		3.8	294

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Diversity Output from Input File: E. erythropoda (April 24, 2006)

Samples	Individuals (computed)	Sobs (Mao Tau)	Singletons Mean	Doubletons Mean	Uniques Mean	Duplicates Mean	ACE Mean	Chao 1 Mean	Chao 1 95% CI Lower Bound	Chao 1 95% CI Upper Bound	Chao 2 Mean	Cole Rarefaction	Shannon Mean	Simpson Mean
1	10.67	10.67	10.78	0	10.78	0	64.64	64.64	28.16	178.67	58.66	10.63	2.37	I
2	21.33	21.25	21.46	0.04	21.46	0.04	243.04	239.25	116.96	518.66	215.06	21.19	3.06	I
3	32	31.75	32	0.16	32	0.16	535.2	498.93	268.65	954.07	447.07	31.67	3.47	I
4	42.67	42.17	41.86	0.48	41.86	0.48	892.56	741.24	426.92	1313.3	663.58	42.07	3.74	I
5	53.33	52.5	51.86	0.76	51.86	0.76	1311.64	983.68	594.47	1653.2	880.23	52.41	3.96	I
6	64	62.75	61.28	1.26	61.28	1.26	1593.4	1112.65	698.94	1795.96	995.97	62.67	4.13	I
7	74.67	72.92	71.3	1.74	71.3	1.74	1864.61	1225.54	795.03	1913.27	1097.49	72.85	4.28	I
8	85.33	83	80.32	2.42	80.32	2.42	1773.69	1105.11	736.21	1682.35	991.51	82.96	4.4	1795.03
9	96	93	90	3	90	3	1488	1094.25	747.91	1623.75	983		4.52	1520

EstimateS (Version 7.5.0), Copyright R. K. Colwell: http://viceroy.eeb.uconn.edu/estimates

Diversity Output from Input File: S. atrovirens (April 24, 2006)

Samples	Individuals (computed)	Sobs (Mao Tau)	Sobs 95% CI Lower Bound	Sobs 95% CI Upper Bound	Sobs SD (Mao Tau)	Sobs Mean (runs)	Singletons Mean	Doubletons Mean	Uniques Mean	Duplicates Mean	ACE Mean	Chao 1 Mean	Chao 1 95% CI Lower Bound	Chao 1 95% CI Upper Bound	Chao 1 SD (analytical)	Chao 2 Mean	Cole Rarefaction	Shannon Mean	Simpson Mean
1	9.5	9.33	7.58	11.09	0.9	9.54	9.38	0.16	9.54	0	50.76	47.41	20.84	137.17	25.88	47.33	9.43	2.24	I
2	19	18.55	15.08	22.01	1.77	18.7	18.26	0.44	18.52	0.18	181.05	152.98	74.08	345.3	64.08	158.46	18.73	2.92	I
3	28.5	27.64	22.51	32.77	2.62	27.62	26.72	0.9	27.2	0.42	356.58	266.96	141.95	529.9	93.74	302.41	27.89	3.3	I
4	38	36.62	29.87	43.38	3.45	36.58	34.98	1.6	35.68	0.9	495.32	332.91	190.24	608.92	102.3	411.8	36.93	3.58	I
5	47.5	45.5	37.15	53.85	4.26	45.36	43.18	2.12	44.2	1.1	606.03	394.44	236.57	683.17	109.88	565.18	45.84	3.8	I
6	57	54.27	44.35	64.19	5.06	54.22	51.26	2.84	52.42	1.68	642.8	433.7	271.37	717.58	110.34	597.64	54.63	3.97	622.17
7	66.5	62.95	51.48	74.42	5.85	62.62	58.8	3.7	60.2	2.3	622.23	463.88	300.61	739.31	108.9	644.95	63.3	4.11	602.25
8	76	71.53	58.53	84.53	6.63	71.28	66.82	4.22	68.34	2.7	684.34	533.68	354.63	826.02	117.4	739.08	71.85	4.24	639.39
9	85.5	80.02	65.5	94.53	7.41	79.5	73.98	5.18	75.7	3.46	653	535.15	365.06	806.65	110.21	707.21	80.3	4.35	618.61
10	95	88.42	72.4	104.44	8.17	88.02	81.68	5.74	83.52	3.9	696.64	592.13	411.09	874.67	115.93	769.85	88.63	4.45	622.09
11	104.5	96.75	79.23	114.27	8.94	96.54	89.42	6.32	91.3	4.44	739.14	646.69	456.03	938.49	120.86	812.79	96.87	4.54	634.24
12	114	105	86	124	9.7	105	97	7	99	5	764.73	687	491.62	981.12	122.79	846.13		4.62	644.1

EstimateS (Version 7.5.0), Copyright R. K. Colwell: http://viceroy.eeb.uconn.edu/estimates

Diversity Output from Input File: None (April 24, 2006)

Samples	Individuals (computed)	Sobs (Mao Tau)	Singletons Mean	Doubletons Mean	Uniques Mean	Duplicates Mean	ACE Mean	Chao 1 Mean	Chao 1 95% CI Lower Bound	Chao 1 95% CI Upper Bound	Chao 1 SD (analytical)	Chao 2 Mean	Cole Rarefaction	Shannon Mean	Simpson Mean
1	9.22	9	8.64	0.14	8.78	0	43.94	41.04	18.07	122.01	22.92	40.03	9.16	2.15	I
2	18.44	17.92	17.24	0.46	17.6	0.1	164.57	134.94	64.93	309.92	57.64	145.06	18.2	2.86	I
3	27.67	26.75	25.54	0.98	26.12	0.4	331.54	224.57	118.8	452.59	80.37	271.07	27.11	3.26	I
4	36.89	35.5	33.48	1.56	34.34	0.7	482.54	290.9	164.63	540.82	91.65	395.84	35.9	3.54	I
5	46.11	44.17	41.46	2.16	42.54	1.08	564.53	338.51	201.94	593.1	96.03	497.26	44.57	3.76	574.61
6	55.33	52.75	49.54	2.76	50.88	1.42	633.59	411.1	255.59	685.76	106.26	619.45	53.11	3.94	649.18
7	64.56	61.25	57.56	3.4	59.14	1.82	622.04	451.71	291.09	724.54	107.52	683.86	61.53	4.09	644.09
8	73.78	69.67	64.78	4.34	66.62	2.5	603.9	466.37	310.02	724.27	103.07	655.36	69.83	4.21	631.92
9	83	78	73	5	75	3	647.4	516	351.17	780.3	107.06	694.67		4.34	680.6

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Diversity Output from Input File: Pooled (June 4, 2006)

NOTE: Chao's estimated CV for Abundance distribution = 0.67. Because the CV > 0.5, Anne Chao recommends that you re-compute Chao1 using the Classic instead of the Bias-Corrected option in the Diversity Settings screen. Then, based on the results with the Classic option, report the larger of Chao1 and ACE as the best estimate for abundance-based richness.

NOTE: Chao's estimated CV for Incidence distribution = 0.651. Because the CV > 0.5, Anne Chao recommends that you re-compute Chao2 using the Classic instead of the Bias-Corrected option in the Diversity Settings screen. Then, based on the results with the Classic option, report the larger of Chao2 and ICE as the best estimate for incidence-based richness.

Samples	Individuals (computed)	S_{obs} (Mao Tau)	Singletons Mean	Doubletons Mean	Uniques Mean	Duplicates Mean	ACE Mean	Chao 1 Mean	Chao 1 95% CI Lower Bound	Chao 1 95% CI Upper Bound	Chao 1 SD (analytical)	Chao 2 Mean	Cole Rarefaction	Shannon Mean	Simpson Mean
1	9.5	9.36	9.22	0.15	9.37	0	50.01	47.35	20.85	136.46	25.73	48.88	9.43	2.21	I
2	19	18.6	18.12	0.4	18.4	0.14	181.81	153.83	74.48	347.21	64.43	169.17	18.74	2.9	I
3	28.5	27.72	26.92	0.72	27.33	0.34	376.72	289.56	153.15	575.66	102.12	334.41	27.92	3.3	I
4	38	36.73	35.56	1.12	36.07	0.68	595.62	418.04	236.12	767.22	129.9	496.1	36.98	3.59	I
5	47.5	45.64	44.03	1.56	44.65	1.02	787.6	531.68	315.55	921.94	149.41	649.2	45.94	3.8	I
6	57	54.45	52.3	2.06	53	1.49	915.95	621.85	383.91	1032.51	160.61	762.12	54.79	3.98	I
7	66.5	63.16	60.38	2.64	61.18	2	997.51	687.71	438.61	1102.73	165.06	849.35	63.55	4.13	I
8	76	71.79	68.38	3.21	69.28	2.52	1036.05	754.05	494.14	1174.47	169.58	920.78	72.21	4.25	I
9	85.5	80.33	76.12	3.8	77.11	3.07	1045.9	800.57	536.97	1216.8	169.83	969.5	80.78	4.36	I
10	95	88.79	83.76	4.43	84.82	3.66	1037.98	838.44	573.87	1247.66	168.63	992.48	89.27	4.46	I
11	104.5	97.17	91.26	5.1	92.42	4.24	1050.93	877.04	610.89	1281.39	168.07	1037	97.67	4.55	728.72
12	114	105.47	98.69	5.8	99.91	4.87	1073.12	909.19	643.4	1306.59	166.45	1057.58	106	4.63	718.18
13	123.5	113.71	105.97	6.53	107.26	5.55	1094.09	937.66	672.71	1328.41	164.75	1082.69	114.25	4.7	699.69
14	133	121.87	113.22	7.22	114.58	6.17	1117.26	969.03	703.76	1355.45	163.9	1111.25	122.43	4.76	693.29
15	142.5	129.97	120.34	7.95	121.76	6.87	1149.33	1004.45	737.46	1388.98	163.99	1139.2	130.55	4.83	690.01
16	152	138.01	127.45	8.68	128.94	7.54	1180.98	1034.91	767.43	1416.24	163.43	1169.11	138.59	4.88	686.2
17	161.5	145.99	134.23	9.51	135.79	8.3	1197.82	1056.16	790.44	1431.62	161.61	1187.88	146.57	4.94	679.53
18	171	153.9	140.99	10.33	142.64	9.05	1219.24	1076.04	812.16	1445.83	159.8	1206.51	154.49	4.99	675.1
19	180.5	161.76	147.76	11.08	149.47	9.72	1261.26	1110.17	844.08	1480.17	160.5	1242.09	162.35	5.03	670.67
20	190	169.56	154.39	11.95	156.16	10.53	1283.65	1129.95	865.37	1495.24	159	1259.05	170.15	5.08	668.68
21	199.5	177.31	161.04	12.75	162.89	11.25	1317.12	1158.13	892.69	1522.16	158.97	1287.4	177.89	5.12	667.9
22	209	185.03	167.64	13.59	169.57	12.02	1351.87	1182.95	917.36	1544.97	158.56	1311.25	185.58	5.16	665.81
23	218.5	193.31	174.05	14.46	176.05	12.79	1379.01	1203.22	938.41	1562.13	157.63	1332.47	193.21	5.2	663.49
24	228	200.3	180.39	15.39	182.48	13.63	1406.25	1219.89	956.65	1574.81	156.28	1348.02	200.79	5.24	661.91
25	237.5	207.85	186.63	16.33	188.78	14.5	1432.73	1237.83	975.68	1589.54	155.24	1363.75	208.32	5.27	660.48
26	247	215.36	192.77	17.3	195.01	15.37	1454.26	1252.14	991.76	1599.89	153.83	1378.36	215.8	5.3	660.5
27	256.5	222.81	198.85	18.28	201.18	16.24	1481.09	1267.12	1008.22	1611.41	152.62	1393.55	223.23	5.34	658.76
28	266	230.22	205.12	19.21	207.51	17.09	1510.53	1287.55	1028.83	1630.1	152.17	1413.15	230.61	5.37	660.82
29	275.5	237.59	211.2	20.18	213.66	17.98	1538.61	1304.52	1046.64	1644.61	151.37	1429.75	237.94	5.4	660.55
30	285	244.91	217.18	21.17	219.73	18.86	1567.53	1320.02	1063.18	1657.51	150.48	1446.16	245.23	5.42	658.96
31	294.5	252.2	223.19	22.13	225.81	19.72	1596.96	1338.14	1081.67	1673.94	149.98	1464.39	252.47	5.45	659.18
32	304	259.44	229.12	23.09	231.81	20.58	1627.2	1355.31	1099.33	1689.33	149.44	1481.56	259.66	5.48	658.36
33	313.5	266.64	235.01	24.1	237.79	21.46	1653.26	1371.11	1115.88	1703.06	148.75	1498.5	266.81	5.5	659.89
34	323	273.8	240.76	25.09	243.6	22.36	1681.14	1385.6	1131.27	1715.4	148	1512.33	273.92	5.53	658.27
35	332.5	280.92	246.32	26.09	249.25	23.23	1707.14	1399.07	1145.69	1726.69	147.23	1526.51	280.98	5.55	655.7
36	342	288	252	27	255	24	1736.36	1417.5	1164	1744.36	147.09	1547.42		5.57	655.18

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14. ABSTRACT

The use of wetlands to effectively remediate aquifers contaminated with chlorinated solvents is an emerging technique, which shows high promise. In order to better understand this process and test its legitimacy, a treatment wetland was constructed at Wright-Patterson AFB, Dayton, Ohio and, in a joint effort with Wright State University (WSU), has previously shown the effective removal of PCE. The purpose of this research was to characterize the soil bacterial community, pre-PCE injection, among three wetland plant species from the sedge family (Cyperaceae) within constructed wetland mesocosms and to identify any bacterial dominance.

Carex comosa, Scirpus atrovirens, and Eleocharis erythropoda were planted in multiple columns (mesocosms) filled with inoculated soils; water flow was through a vertical up-flow design representative of a ground water-fed wetland. DNA extractions were made from soil samples taken at each three depths. 16S rDNA polymerase chain reaction (PCR), cloning, plasmid DNA isolation, and sequencing were used to characterize the bacterial community.

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